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PROCEEDINGS

OF THE

ROYAL SOCIETY OF LONDON

SERIES B

CONTAINING PAPERS OF A BIOLOGICAL CHARACTER

VOL. LXXXI.

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PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

Address of the President, Lord Rayleigh, O.M., D.C.L., at the Anniversary Meeting on November 30, 1908.

Since the last Anniversary the Society has sustained the loss of eighteen Fellows and four Foreign Members.

The deceased Fellows are:—

The Right Hon. Lord Kelvin, died December 17, 1907.
Sir Alfred Baring Garrod, died December 28, 1907.
Robert Lewis John Ellery, died January 14, 1908.
Prof. James Bell Pettigrew, died January 31, 1908.
William Ashwell Shenstone, died February 3, 1908.
Sir John Denis Macdonald, died February 7, 1908.
Lieutenant-General Sir Richard Strachey, died February 12, 1908.
Dr. William Edward Wilson, died March 6, 1908.
Dr. Henry Clifton Sorby, died March 9, 1908.
Sir John Eliot, died March 17, 1908.
The Duke of Devonshire, died March 24, 1908.
Dr. James Bell, died March 31, 1908.
Colonel Andrew Wilson Baird, died April 2, 1908.
Sir John Evans, died May 31, 1908.
Lord Blythswood, died July 8, 1908.
Arthur Lister, died July 20, 1908.
The Earl of Rosse, died August 29, 1908.
Prof. William Edward Ayrton, died November 8, 1908.

The deceased Foreign Members are:—

Pierre Jules César Janssen, died December 23, 1907.
Franz von Leydig, died April, 1908.
Henri Becquerel, died August 25, 1908.
Éleuthère Élie Nicolas Mascart, died August 26, 1908.

The list of deaths this year is exceptionally heavy, and includes the name of one of the most eminent scientific men of our generation, who occupied the Presidency of this Society from 1890 to 1895—I refer, of course, to Lord Kelvin.

We are fortunate in having secured for our 'Proceedings' a review of Kelvin's life and work, written by one who is especially well qualified for the difficult task. I do not doubt that Professor Larmor is right in placing in the forefront of that work those fundamental advances in Thermodynamics which date from the middle of the last century. It was Kelvin who first grasped the full scope of the principle known as the Second Law, a law which may indeed well be considered to stand first in order of importance, regarded from the point of view of man's needs and opportunities. It would be futile to attempt here a re-survey of the ground covered by Professor Larmor.

My acquaintance with Kelvin was limited, until about 1880, a time when I was occupied with measurements relating to the electrical units, and received much appreciated encouragement. From then onwards until his death I enjoyed the privilege of intimacy and, needless to say, profited continually from his conversation, as I had done before from his writings. Our discussions did not always end in agreement, and I remember his admitting that a certain amount of opposition was good for him. Such discussions often invaded the officers' meetings during the time that we were colleagues, not always to the furtherance of the Society's business. But I must not linger over these reminiscences, interesting as they are to me. We shall never see his like.

By the death of Sir Richard Strachey we have lost a man well known to the senior Fellows, who served repeatedly upon the Council and whose advice was always valued. He was a born administrator; and by his work in India and afterwards at the Meteorological Office he rendered splendid service.

Dr. Sorby's researches extended over many fields, and in several of them he was a pioneer. I suppose that his greatest achievement was the introduction of the method in which thin slices of rock are examined under the microscope. Among his many interesting observations are those upon the retardation of freezing in capillary tubes. It appears that the walls exercise an influence at distances much greater than those usually regarded as molecular—evidence apparently of structure upon an extended scale. Dr. Sorby belonged to a class on whom England has special reason to congratulate herself, men who pursue science unprofessionally. The names of Cavendish, Young, Joule, and Darwin at once suggest themselves. It is to be feared that specialisation and the increasing cost and complication of experimental appliances are having a prejudicial effect in this regard. On the other hand, the amateur is not without advantages which compensate to some extent. Certainly, no one who has the root of the matter in him should be deterred by fears of such difficulties, and the example of Sorby suffices to show how much is open to ingenuity unaided by elaborate appliances.

The name of Sir John Evans must not pass without special notice. There are few in recent years to whom the Society has been more indebted. Many of our Fellows hardly realise how important and laborious are the services rendered in the office of Treasurer. Evans' scientific attainments, his knowledge of the world and of business, and his personal characteristics specially qualified him for office. An appreciation, signed by well-known initials, has recently appeared in our 'Proceedings.'

On the Foreign List also the losses are heavy. We have especially to condole with our colleagues in France upon the havoc caused by death within the last year or two. Janssen, and Mascart, who was much missed at the recent Electrical Conference, had reached a full age. But Becquerel was in the full tide of life, and we had hoped to learn much more from him; as the discoverer of radio-activity, he had opened up inquiries whose significance seems ever on the increase. Science has lost a leader; his friends and the world a charming personality.

During the time that I was Secretary, and so concerned with the passing of mathematical papers through the Press, I was much struck with the carelessness of authors in the arrangement of their manuscript. It is frequently forgotten that a line of print in the 'Transactions' and in the new form of the 'Proceedings' will hold much more than a line of ordinary manuscript, unless, indeed, the handwriting is exceptionally small. Unless the authors' indications were supplemented, it frequently occurred that several lines of print were occupied by what might equally well, and in my judgment much better, be contained in one line. Even practised writers would do well, when they regard their manuscript as complete so far as regards matter and phrasing, to go over it again entirely from the point of view of the printing. In this way much expense and space would be spared, and the appearance of the printed page improved. Professor Larmor has drawn up a paper which has received the sanction of the Council and is appended to this Address, and will, it is hoped, be of service at once to authors and to the Society. (The paper is printed in Series A only.)

Apart from questions of printing, the choice of symbols for representing mathematical and physical quantities is of some importance, and is embarrassed by varying usages, especially in different countries. A Committee now sitting is concerned with the selection of symbols for electrical and magnetic quantities, but the question is really much wider. One hesitates to suggest another international conference, and perhaps something could be done by discussion in scientific newspapers. Obviously some give and take would be necessary. When the arguments from convenience are about balanced, appeal might be made to the authority of distinguished men, especially of those who were pioneers in the definition and use of the quantity to be represented. As an example of the difficulties to be faced, I may instance the important case of a symbol for refractive index. In English writings the symbol is usually μ, and on the Continent n. By the early optical writers it would seem that no particular symbol was appropriated. In 1815* Brewster has m. The earliest use of μ that I have come across is by Sir John Herschel,† and the same symbol was used by Coddington (1829) and by Hamilton (1830), both distinguished workers in optics. On the other hand, n was employed by Fraunhofer (1815), and his authority must be reckoned very high. As regards convenience, I should suppose that the balance of advantage would incline to μ, since n is wanted so frequently in other senses. Another case in which there may be difficulties in obtaining a much to be desired uniformity is the symbol for electrical resistance.

On a former occasion I indulged in comment upon the tendency of some recent mathematics, which were doubtless understood as the mild grumbling of an elderly man who does not like to see himself left too far behind. In the same spirit I am inclined to complain of what seem unnecessary changes in mathematical nomenclature. In my youth, by a natural extension of a long established usage relative to equations, we spoke of the *roots* of a function, meaning thereby those values of the argument which cause the *function* to vanish. In many modern writings I read of the *zeroes* of a function in the same sense. There may be reasons for this change; but the new expression seems to need precaution in its use; otherwise we are led to such flowers of speech as "zeroes with real part positive," which I recently came across.‡

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* 'Phil. Trans.,' 1815.

† 'Phil. Trans.,' 1821, p. 230.

‡ 'Proc. Math. Soc.,' vol. 31, p. 266.

But though I may use a little my privilege of grumbling over details, I hope I shall not be misunderstood as undervaluing the progress made in recent years, which, indeed, seems to me to be very remarkable and satisfactory, regarded from the scientific point of view. On the other hand I cannot help feeling misgivings as to the suitability of the highly specialised mathematics of the present day for a general intellectual training, and I hope that a careful watch may be maintained to check, in good time, any evil tendencies that may become apparent.

Among the notable advances of the present year is the liquefaction of helium by Professor Onnes of Leiden. It is but a few years since Sir J. Dewar opened up a new field of temperature by his liquefaction of hydrogen, and now a further extension is made which, if reckoned merely in difference of temperature, may appear inconsiderable, but seen from the proper thermodynamical standpoint is recognised to be far-reaching. The exploration of this new field can hardly fail to afford valuable guidance for our ideas concerning the general properties and constitution of matter. Professor Onnes' success is the reward of labours well directed and protracted over many years.

The discovery and application by Rutherford and Geiger of an electrical method of counting the number of α-particles from radio-active substances constitutes an important step, and one that appears to afford better determinations than hitherto of various fundamental quantities. It would be of interest to learn what interpretation is put upon these results by those who still desire to regard matter as homogeneous.

Another very interesting observation published during the year is that of Hale upon the Zeeman effect in sun-spots, tending to show that the spots are fields of intense magnetic force. Anything which promises a clue as to the nature of these mysterious peculiarities of the solar surface is especially welcome. Until we understand better than we do these solar processes, on which our very existence depends, we may do well to cultivate a humbler frame of mind than that indulged in by some of our colleagues.

A theoretical question of importance is raised by the observations of Nordmann and Tikhoff showing a small chromatic displacement of the phase of minimum brightness in the case of certain variable stars. The absence of such an effect has been hitherto the principal argument on the experimental side for assuming a velocity of propagation in vacuum independent of frequency or wave-length. The tendency of the observations would be to suggest a dispersion in the same direction as in ordinary matter, but of almost infinitesimal amount, in view of the immense distances over which the propagation takes place. Lebedew has pointed out that this conclusion may be evaded by assuming an asymmetry involving colour in the process by which the variability is brought about, and he remarks that although the dispersions indicated by Nordmann and Tikhoff are in the same direction, the amounts calculated from the best available values of the parallaxes differ in the ratio of 30 to 1. In view of this discrepancy and of the extreme minuteness of the dispersion that would be indicated, the probabilities seem at the moment to lie on the side of Lebedew's explanation; doubtless further facts will be available in the near future.

I cannot abstain from including in the achievements of the year the remarkable successes in mechanical flight attained by the brothers Wright, although the interest is rather social and practical than purely scientific. For many years, in fact ever since I became acquainted with the work of Penaud and Wenham, I have leaned to the opinion that flight was possible as a *feat*. This question is now settled, and the tendency may perhaps be to jump too quickly to the conclusion that what can be done as a feat will soon be possible for the purposes of daily life. But there is a very large gap to be bridged over; and the argument urged by Professor Newcomb and based on the principle of dynamical similarity, that the difficulties must increase with the scale of the machines, goes far to preclude the idea that regular ocean service will be conducted by flying machines rather than by ships. But, as the history of science and invention abundantly proves, it is rash to set limits. For special purposes, such as exploration, we may expect to see flying machines in use before many years have passed.

The Report of the National Physical Laboratory for the year again indicates remarkable growth. The various new buildings, which have been erected and equipped during recent years at a cost of about £33,000, are now occupied; and the result is that both researches and test work can be carried out with much greater ease and efficiency than previously. The Executive Committee in charge of the Laboratory is indebted in the first instance to H.M. Government, and then to the numerous friends whose assistance has made this possible. At the same time, the needs for buildings are not nearly satisfied. There has been during the year a very marked and important growth in the demand by manufacturers and others for assistance in metallurgical enquiries, which require investigations, frequently of a very complex character; and with the present accommodation for much of the Metallurgical Department this demand is difficult to satisfy. Thanks in great measure to the Goldsmiths' Company, the chemical side of this department is well provided for; but new buildings for the other branches of metallurgy are an urgent want.

The Report of the Treasury Committee of Inquiry referred to in the address of last year was communicated by the Treasury to the Royal Society, with the intimation that Their Lordships accept the recommendations of the Committee, and trust that the Royal Society may see their way to do the same. In their reply the President and Council, with the concurrence and advice of the Executive Committee of the Laboratory, expressed their readiness to use their best endeavours to carry the Report into effect. The Report has since been presented to Parliament.

The buildings of the Magnetic Observatory at Eskdalemuir are now occupied; but, unfortunately, difficulty has arisen in making the magnetograph rooms which are underground completely watertight, and the recording apparatus is not yet properly installed.

The third and fourth volumes of 'Collected Researches' of the Laboratory have been published during the year, and testify to the vigorous scientific activities of the staff. The third volume is occupied chiefly with the account of the prolonged series of experiments on electric units carried out at the Laboratory by Prof. Ayrton, Mr. Mather, Dr. Lowry, and Mr. Smith. These researches proved of great value in the discussions at the International Conference on Electric Units, for which recently the Society provided accommodation and entertainment at the request of the Government.

The progress of the 'Royal Society Catalogue of Scientific Papers' has advanced a definite stage during the year, through the publication by the Cambridge University Press of the Index Volume of Pure Mathematics for the Nineteenth Century. Owing to the magnitude of the material to be indexed in the several sciences, it has been necessary to adopt drastic measures of compression, and the 40,000 entries involved in the present section have thus been condensed into one royal octavo volume of some 700 pages. An essential element in this saving of bulk has been the grouping of titles within each heading so as to avoid reprinting the leading words. It was, perhaps, inevitable that this device would occasionally be mistaken for an attempt at organic classification within the limits of the main headings, which are substantially those of the yearly 'International Catalogue of Scientific Literature.' This had, indeed, been foreseen in the preface of the volume. As regards new actual sub-headings which have been introduced occasionally, the Committee remark that "These minor classifications, being often made mechanically on the basis of the explicit mention of the sub-heading, are not to be taken as exhaustive; cognate entries may be found elsewhere under the same main heading. The unit of classification is thus the complete numbered heading."

The Committee of the Catalogue have indeed been fully conscious throughout of the difficulties of the task which they supervise; and it must be gratifying to the Director of the Catalogue and his staff to have the support of high authorities, not confined to this country, in their decision that in so extensive an undertaking practical feasibility must be the aim rather than an elusive theoretical perfection. One advantage, at any rate, will accrue from bringing out a single volume well in advance, in that the Committee will be able to profit in the future work from the experience they have acquired.

Through the kindness of Dr. Schuster I had the opportunity of submitting to the Council, before the expiry of my term of office, a generous proposal which he makes for instituting a fund of £1500, the interest of which is to be applied to pay the travelling expenses of delegates of the Society to the International Association of Academies. Dr. Schuster felt that the absence of such a provision laid a burden upon delegates, and might operate to limit the choice of the Society. I was empowered by the Council to convey their cordial thanks to Dr. Schuster, and I have now the pleasure of making his benefaction known to the Society at large.

In taking leave of the honourable office which I have occupied for three years, I desire to thank the Society and especially my colleagues, the officers, for the consideration which they have uniformly shown me. All the omens indicate that the Society will be represented by one well versed in its affairs, and whose scientific distinction and wide experience justify the highest hopes for his tenure of the chair.

MEDALLISTS, 1908.

Copley Medal.

The Copley Medal is awarded to Dr. Alfred Russel Wallace, F.R.S.

It is now sixty years since this distinguished naturalist began his scientific career. During this long period he has been unceasingly active in the prosecution of natural history studies. As far back as 1848 he accompanied the late Henry Walter Bates to the region of the Amazon, and remained four years there, greatly enriching zoology and botany, and laying at the same time the basis of that wide range of biological acquirement by which all his writings have been characterised. From South America he passed to the Malay Archipelago and spent there some eight fruitful years. It was during his stay in that region that he matured those broad views regarding the geographical distribution of plants and animals which on his return to this country he was able to elaborate in his well-known classic volumes on that subject. It was there, too, amid the problems presented by the infinite variety of tropical life, that he independently conceived the idea of the theory of the origin of species by natural selection which Charles Darwin had already been working out for years before. His claims to the admiration of all men of science were recognised by the Royal Society forty years ago, when, in 1868, a Royal Medal was awarded to him. Again, when in 1890, the Darwin Medal was founded, he was chosen as its first recipient. He is still full of mental activity and continues to enrich our literature with contributions from his wide store of experience and reflection in the domain of Natural History. As a crowning mark of the high estimation in which the Royal Society holds his services to science, the Copley Medal is now fittingly bestowed on him.

Rumford Medal.

The Rumford Medal is awarded to Prof. H. A. Lorentz, For. Mem. R.S.

Prof. Hendrik Antoon Lorentz, of Leiden, has been distinguished during the last quarter of a century by his fundamental investigations in the principles of the theory of radiation, especially in its electric aspect. His earliest memoirs were concerned with the molecular equivalents which obtain in the refractive (and dispersive) powers of different substances; in them he arrived at formulæ that still remain the accepted mode of theoretical formulation of these phenomena. The main result, that $(\mu^2-1)/(\mu^2+2)$ is proportional jointly to the density of distribution of the molecules, and to a function of the molecular free periods and the period of the radiation in question, rests essentially only on the idea of propagation in some type of elastic medium; and thus it was reached simultaneously, along different special lines, by H. A. Lorentz originally from Helmholtz's form of Maxwell's electric theory, and by L. Lorenz, of Copenhagen, from a general idea of propagation after the manner of elastic solids.

The other advance in physical science with which Prof. Lorentz's name is most closely associated is one of greater precision, the molecular development of Maxwell's theory of electro-dynamics. This subject was never entered upon by Maxwell himself, on the ground, probably, that the general relations of the æther, and in particular their dynamical bearings, offered a definite field which must be fully probed and explored before the uncertainties connected with molecular complexity became ripe for effective detailed treatment. But the theoretical difficulties connected with the simple law of the astronomical aberration of light, and particularly with the entire absence of any effect of the Earth's uniform motion in space on terrestrial phenomena involving radiation, had more recently rendered this problem urgent. Following on various purely optical papers on the phenomena of moving bodies, Prof. Lorentz, in 1892, elaborated a general molecular treatment in the memoir "La Théorie Electro-magnétique de Maxwell, et son Application aux Corps Mouvants," which appeared in the 'Archives Néerlandaises,' and contains substantially the main root ideas of the subject. In 1905 it was re-expounded with further development in a tract entitled "Versuch einer Theorie der Electrischen und Optischen Erscheinungen in Bewegten Körpern," the main feature being the elimination of the dynamical element in the previous discussion in favour of a formulation by a system of abstract equations, after the way first set out by Maxwell himself as a summary of his final definite results as distinct from the formative ideas underlying them, and afterwards brought into prominence by the expositions of Heaviside and Hertz.

By these writings Prof. Lorentz has taken a predominant place in the modern evolution of electric and optical theory. He has since been active in special applications, of which the best known has been his theoretical prediction of the physical features of the alteration of the lines of the spectrum in a magnetic field, which had been discovered and has since been developed by his colleague Zeeman.

ROYAL MEDALS.

The assent of His Majesty the King, our Patron, has been graciously signified to the following awards of the Medals presented annually by him to the Society.

A Royal Medal to Prof. John Milne, F.R.S., for his work on Seismology. In 1875, Dr. Milne accepted the position of Professor at Tokyo, which was offered to him by the Imperial Government of Japan. His attention was almost immediately attracted to the study of earthquakes, and he was led to design new forms of construction for buildings and engineering structures with a view to resisting the destructive effects of shocks. His suggestions have been largely adopted, and his designs have been very successful for the end in view. Incidentally he studied the vibrations of locomotives, and showed how to obtain a more exact balancing of the moving parts, and thus to secure smoother running and a saving of fuel. Here again his suggestions were accepted, and his work was recognised by the Institution of Civil Engineers.

He next devoted himself to the study of artificial shocks produced by the explosion of dynamite in borings. He then studied actual shocks as observed at nine stations connected by telegraph wires. A seismic study of Tokyo, and subsequently of the whole of northern Japan, followed. In this latter work he relied on reports from 50 stations. The Government then took up the matter, increased his 50 stations to nearly 1000, and founded a Chair of Seismology for Mr. Milne. It is due to his energy, skill, and knowledge that the Japanese School of Seismology stands as the first in the world.

While still in Japan he attempted to obtain international co-operation through the representatives of 13 nationalities. This first effort failed; but subsequently, on his return to England in 1895, he succeeded, and reports are now received by him from some 200 stations furnished with trustworthy instruments, and scattered all over the world. On his return to England he at once established his own observatory at Shide, in the Isle of Wight, and the work has been carried on continuously from that time up to now, mainly by his own industry and resources.

In Great Britain we owe everything in seismology to the British Association. Their Committee was founded in 1880, and since that date Milne has been the moving spirit in the long career of its activity. He has been the author of 29 annual reports, and these form in effect a history of the advance of seismology since it has been recognised as a definite branch of science.

The knowledge which we have now acquired as to the internal constitution of the earth is more due to Milne than to any other man.

The work of Dr. Henry Head, F.R.S., on which is founded the award of the other Royal Medal, forms a connected series of researches on the Nervous System (made partly in conjunction with Campbell, Rivers, Sherren, and Thompson), published for the most part in 'Brain' at various times since 1893 up to the present date, and constituting one of the most original and important contributions to neurological science of recent times.

His first paper (1893), founded on minute and laborious clinical investigation, established in a more precise manner than had hitherto been done the relations between the somatic and visceral systems of nerves. He confirmed from the clinical side the experimental researches of Sherrington on the distribution of the posterior roots of the spinal nerves.

An inquiry into the pathology of Herpes Zoster (1900), which he proved abundantly to be due to inflammation of the posterior root ganglia, indicated that the areas of referred pain in visceral disease corresponded specially with the distribution of the fibres of the posterior roots subserving painful cutaneous sensibility.

Continuing his investigations on the peripheral nerves, partly by experiments on himself, in conjunction with Rivers, and partly by examination of cases of accidental injuries to nerves, Head was led to formulate (1905) an entirely novel conception and differentiation of the functions of the peripheral nerves, and of the paths for the respective forms of sensibility which they convey—epicritic, protopathic, and deep sensibility. This is generally regarded by neurologists as a research of quite exceptional originality and ability.

Following the course of afferent impulses, Head next showed (1906) that the sensory paths of the peripheral nerves at their first synaptic junction with the spinal cord become re-arranged, and ascend in different relations in certain definite tracts.

DAVY MEDAL.

The Davy Medal is awarded to Prof. William Augustus Tilden, F.R.S.

The researches of Prof. Tilden extend into many domains. His work on the specific heats of the elements in relation to their atomic weights, described to the Society in the Bakerian Lecture for 1900 and in two later papers published also in the 'Philosophical Transactions,' was of high theoretical importance. The employment of liquid oxygen as an ordinary laboratory reagent, rendered possible by the researches of Dewar and others, enabled Prof. Tilden to test the validity of Dulong and Petit's Law and of Neumann's Law over a much wider range of temperature than was possible before, and gave a truer estimate of the nature of their validity.

In the region of organic chemistry, he has carried out important researches on the terpenes, such as that on the hydrocarbons from *Pinus sylvestris*, on terpin and terpinol, and on limettin.

In inorganic chemistry, his investigation on *aqua regia* and on nitrosyl chloride are especially noteworthy. He has assisted much in clearing up many points with regard to *aqua regia* about which obscurity remained. His introduction of nitrogen peroxide and especially of nitrosyl chloride as reagents has proved, in his own hands and in those of other workers, to be of very high value.

DARWIN MEDAL.

The Darwin Medal is awarded to Prof. August Weismann for his contributions to the study of evolution. He was one of the early supporters of the doctrine of evolution by means of natural selection, and wrote in support of the Darwinian theory in 1868. His great series of publications from that date onward must always remain a monument of patient inquiry. In forming an estimate of his work it does not seem essential that we should decide on the admissibility of his germ-plasm theory. It is in like manner unimportant that he was, in certain respects, forestalled by Galton, and that his own views have undergone changes. The fact remains that he has done more than any other man to focus scientific attention on the mechanism of inheritance. By denying the possibility of somatic inheritance, he has compelled the world to look at this question with a closeness of criticism that is wanting in all earlier inquiries. In the opinion of what is perhaps the majority of naturalists, he has achieved much more than this—he has convinced them that the solution of the problem of evolution must be sought along the lines of his doctrine of germinal continuity. Thus the preformist's point of view, for which he has done so much, forms the basis on which Mendelians and Mutationists are at work.

Weismann's work was highly estimated by Mr. Darwin. Thus he writes, in 1875 ('More Letters,' i, 356), of Weismann's paper on Seasonal Dimorphism: "No one has done so much as you on this important subject, *i.e.*, on the causes of variation." Again ('Life and Letters,' iii, 198): "I have been profoundly interested by your essay on 'Amblystoma,' and think you have removed a great stumbling block in the way of evolution." And, once more, in January, 1877 ('Life and Letters,' iii, 231), Darwin wrote of Weismann's 'Studien zur Descendenzlehre': "They have excited my interest and admiration in the highest degree, and whichever I think of last seems to me the most valuable."

HUGHES MEDAL.

The Hughes Medal is awarded to Prof. Eugen Goldstein.

Prof. Goldstein was one of the early workers on the modern detailed investigation of the electric discharge in rarefied gases, and by long continued researches has contributed substantially to the systematic analysis of the complex actions presenting themselves in that field. Of these researches may be mentioned his observations of the effect of magnetic force on striations, of the phosphorescence produced by the cathode rays, and of the reflection of cathode rays.

By his discovery of the so-called Kanal-Strahlen, or positive rays, he has detected an essential feature of the phenomenon, which, in his own hands and in those of other workers, has already thrown much needed light on the atomic transformations that are involved.

A Trypanosome from Zanzibar.

By Colonel Sir DAVID BRUCE, C.B., M.B., F.R.S., D.Sc., LL.D., Army Medical Service, and Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps.

(Received September 18,—Read November 26, 1908.)

(From the Laboratory of the Royal Army Medical College, London.)

[PLATES 1 AND 2.]

About the middle of April, 1908, Dr. J. Rose Bradford, F.R.S., had handed over to him by Dr. Edington, F.R.S.E., a rabbit whose blood contained a trypanosome. Dr. Edington stated that he had inoculated the rabbit with blood from a horse he found at Zanzibar suffering from some obscure disease. This rabbit was handed over to one of us (D. B.) by Dr. Bradford for the purpose of keeping the strain alive and, if possible, identifying the species of trypanosoma.

The following notes have since been received from Dr. Edington. The trypanosome was found at Zanzibar, where no trypanosome has formerly been known. It occurred in a horse in a stable among others, of which none were infected. The animal was old, and had been many years in the place. At death the symptoms were like those in surra and nagana, but the spleen was not enlarged, nor was it coloured abnormally. The usual œdema was apparent and most marked in the sheath, up the abdomen, in the chest, and down the posterior limbs.

Dr. Edington inoculated a horse, an ox, and a goat successfully. The disease ran a sub-acute form in the original horse, but in the inoculated one it seemed rather more acute. Inoculated on February 18, trypanosomes were seen in its blood on the 25th, and by March 1 the sheath was swollen. There was no real fever (102°·2 F.) until February 28, so that in this case the appearance of parasites preceded the fever. On March 7 it had greatly recovered, œdema had subsided, and the weakness of the preceding few days was recovered from. Dr. Edington left on March 8, and fears the animal was destroyed, as they had no further vote for funds for food, etc.

A young ox, inoculated on February 15, showed trypanosomes on the 27th. It had fever fairly high, but had recovered before he left, and trypanosomes were exceedingly few. A goat showed high fever, but its blood never showed trypanosomes at any time, although Dr. Edington hunted with very great thoroughness.

Two rabbits were inoculated, one subcutaneously and one intraperitoneally. The former was sent to Dr. Mesnil from Marseilles, but it has shown nothing. It was twice inoculated with big doses, one from a horse and one from an ox. The other Dr. Edington handed over to Dr. Bradford, and from this rabbit the trypanosome under consideration was obtained and studied.

On examining the rabbit's blood, the trypanosome was found to be a small one, with poorly-developed undulating membrane, and no free flagellum. The average length was only 13·5 microns.

Although it is impossible in some cases to name the trypanosomes from their shape and size alone, still it is evident that a trypanosome of this size, with no free flagellum, cannot be *Trypanosoma brucei, evansi, gambiense*, or several other species which need not be enumerated. The names of such small trypanosomes as *Trypanosoma nanum* (Laveran), *Trypanosoma congolense* (Broden), or *Trypanosoma dimorphon* (Dutton and Todd), at once occur to the mind.

No doubt the tendency in naming these hæmatozoa is to multiply unnecessarily the number of species. But, on the other hand, it is just as great a mistake to lump too many species together, as has been done. If there is some well-marked difference in two trypanosomes, even if alike in shape, such as their power of setting up disease in certain animals, their mode of spreading from the sick to the healthy—it may be in one by tsetse flies, in another by stomoxys, or tabanus, or by other means—then, naturally, it is of great practical use to distinguish them by different specific names.

Again, it might be argued, that if two trypanosomes were different morphologically, but had the same effect on animals, the same distribution and the same carrier, then the two varieties for practical purposes might be included in the same species.

For example, when we have to do with *Trypanosoma gambiense* we at once know that man is susceptible, that the carrier is *Glossina palpalis*, and that we must keep ourselves out of the area of distribution of this fly if we would escape infection. Theories in regard to the spread of sleeping sickness by mosquitoes, stomoxys, fleas, sexual intercourse, and such like, may, for practical purposes, be ignored. If it is *Trypanosoma brucei*, then we know man is not susceptible, but that we must keep our horses, cattle, and dogs out of the area of distribution of *Glossina morsitans*.

The three most important questions to be borne in mind, in classifying trypanosomes, are, what animals are they capable of infecting, the gravity of the infection, and, thirdly, what is the carrier? To these may be added the morphology of the trypanosome, its cultural characteristics, if any, and, if possible, cross-inoculation experiments. If these several facts could be set down for each trypanosome encountered in Africa, then some classification of the African species might be attempted. But it is only for a few species, such as *Trypanosoma gambiense* and *Trypanosoma brucei*, that we have all these data. Take, for example, the case of *Trypanosoma congolense* (Broden) and *Trypanosoma dimorphon* (Dutton and Todd)—most important trypanosome diseases. Laveran thinks they are distinct on account of a cross-inoculation experiment, but Broden himself, Rodhain, and Dutton and Todd all seem to lean to their really being one and the same species. With the data at our disposal at present it is impossible to come to a definite decision.

At the present time the classification of the pathogenic trypanosomes is in a state of chaos, and we have no desire to add to the confusion. Nevertheless, we think it will be well to give a description of Dr. Edington's trypanosome, as far as we have been able to study it, in view of the fact that we are starting at once for Uganda to continue the investigation of sleeping sickness.

MORPHOLOGY OF DR. EDINGTON'S TRYPANOSOME.

A. *Living, unstained.*

Dr. Edington's trypanosome in the fresh condition, as seen in a drop of blood from an infected guinea-pig or rat, appears short and stumpy in outline, about twice the diameter of the red blood corpuscles, among which it slowly moves, with, as a rule, its rapidly-vibrating flagellum in front. The posterior or non-flagellar extremity appears blunt and rounded off abruptly, while the anterior tapers off to a fine point. In the fresh preparation the undulating membrane is not much in evidence, though sometimes it can be seen thrown into waves. The contents of the cell are homogeneous, except for a small refractile body at the posterior extremity, which is evidently the micro-nucleus.

B. *Fixed and stained.*

Method of staining.—The method used for fixing and staining the trypanosomes is usually as follows. The blood-film while still moist is exposed to the vapour of a 4-per-cent. solution of osmic acid in distilled water, to which a drop of glacial acetic acid has been added, for 45 seconds. The cover-glass is then transferred to absolute alcohol for from five minutes to half an hour. It is then passed through grades of alcohol from 80 per cent. to 10 per cent. in distilled water. Twenty-five drops of Giemsa's stock stain (Grübler's) are now mixed with 25 c.c. of distilled water. The films are placed in this, face downwards, for 8 to 12 hours, then washed in distilled water, and rinsed quickly in solution of orange tannin (orange G. 1 per cent., tannin 5 per cent., in distilled water). When sufficiently decolorised, the films are washed in distilled water, dehydrated by passing through acetone, cleared in xylol, and mounted in canada balsam.

Dr. Edington's trypanosome when stained in this way appears of a pale puce colour with reddish-purple nucleus and micro-nucleus. The following detailed description must be understood to refer to this trypanosome as found in the blood of the white rat.

Length.—It is no easy matter to measure these small irregularly-shaped bodies, and doubtless the method of measurement used will govern to some extent the result. The method used by us is simply to draw a sharp outline of the trypanosome by means of a Zeiss camera lucida, at a magnification of 2000 diameters, and then to measure along the middle line of the body by means of a pair of fine compasses, the points of which are separated 2 mm. Each step the compass takes is therefore equal to 1 micron. Twenty trypanosomes, taken as they come, are measured in this way in each specimen, and an average of the 20 measurements taken. The following table gives some of the results:—

Dr. Edington's Trypanosome.

No. of experiment.	Day of disease.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
134, mouse	9	Giemsa	15·3	20·0	13·0
69, rat	30	Leishman	13·0	16·0	10·0
„	30	Giemsa	13·9	17·0	10·0
„	30	Methyl green	13·4	18·0	10·0
„	30	Giemsa	15·0	18·0	13·0
„	30	Leishman	13·5	17·0	11·0
84, rabbit	22	Giemsa	13·0	16·0	9·0
Guinea-pig	18	„	12·5	16·0	8·0
166, dog	14	„	13·0	16·0	10·0
		Average	13·6	17·1	10·4

For purposes of comparison measurements of *Trypanosoma dimorphon* and *Trypanosoma congolense* are given in the following tables:—

No. of experiment.	Day of disease.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
		Trypanosoma dimorphon.			
Mouse (Laveran and Mesnil)	?	Giemsa	13·8	17·0	12·0
116, rat (Breinl)	15	"	13·8	16·0	11·0
" "	9	Leishman	11·3	14·0	9·0
" "	9	"	12·6	15·0	11·0
Dog (Harvey, Sierra Leone)	?	"	12·3	15·0	9·0
Cow (Smith, Sierra Leone)	?	"	12·4	15·0	10·0
		Average	12·5	15·3	10·3
		Trypanosoma congolense.			
142, mouse	7	Giemsa	12·8	15·0	10·0
143, mouse	5	Leishman	11·5	14·0	10·0
152, rat	11	Giemsa	11·6	15·0	9·0
"	11	"	11·5	14·0	10·0
154, rat	19	"	12·6	17·0	10·0
		Average	12·0	15·0	9·8

Breadth.—On an average the breadth at the widest part is 3 microns.

Shape.—Dr. Edington's trypanosome when stained is seen to be of a short and stumpy shape, somewhat reminding one of a miniature electric eel. The posterior extremity is, as a rule, blunt, or rounded or obtuse-angled, but sometimes, though rarely, it is prolonged into a sharp beak-like process. The anterior end tapers more or less, and ends in a short stout flagellum. The undulating membrane is narrow but distinct. The flagellum arises at or near the micro-nucleus and passes along the edge of the undulating membrane. There is no free flagellum, the protoplasm of the cell and the undulating membrane extending as far as the tip of the flagellum.

Contents of Cell.—The protoplasm, which is stained a pale puce colour, is homogeneous in structure.

Nucleus.—The nucleus is oval in shape, about 2·5 microns in length, and is situated at the centre of the trypanosome.

Micro-nucleus.—The micro-nucleus, centrosome, or kineto-nucleus, is small, round, or rod-shaped, and is situated close to the posterior extremity. It stains more deeply than the nucleus.

Undulating Membrane.—The undulating membrane is narrow. As a rule it is straight and simple, and does not show much tendency to be thrown into folds.

Flagellum.—The flagellum stains intensely. It is well marked, and does not project beyond the protoplasm of the cell and the undulating membrane. Sometimes, in faintly-stained specimens, there is the appearance of a slight projection of the flagellum beyond the body; but, speaking broadly, this species of trypanosome may be said to have no free flagellum.

The conclusion to be drawn from a study of the morphology of Dr. Edington's trypanosome, *Trypanosoma dimorphon,* and *Trypanosoma congolense,* is, that the two first resemble each other very closely, whereas *Trypanosoma congolense* seems to be of a somewhat shorter and stouter form. It will also be seen that in the strain of *Trypanosoma dimorphon* used there is only one form, and that, the short or tadpole form described by Dutton and Todd. With regard to this, it may be of interest to quote some remarks of Dr. Breinl, to whom I am obliged for his courtesy in sending me this strain. He writes:—" With regard to *Trypanosoma dimorphon,* you are aware that some remarkable change has occurred in the strain between the time Drs. Dutton and Todd brought it back from Africa and we started work on it here. Whereas Drs. Dutton and Todd describe the long flagellated forms with the free flagella, Thomas and myself, Laveran and Mesnil, could not see these forms with a thin body and a long flagellum. The strain I send you in a rat is the original strain."

It is difficult to understand how this change in morphology has been brought about. It may be that Dutton and Todd were dealing with a double infection, of which one has died out. This point will require to be investigated on the spot.

Another matter for consideration is whether this name *Trypanosoma dimorphon* should be adhered to. It certainly seems a misnomer when applied to the strain figured above. If it should be decided to drop it, I think the compliment should be paid to Dr. Todd of naming it after him.

Inoculation Experiments on various Species of Animals.

The animals, in which the effect of the inoculation of Dr. Edington's trypanosome has been studied, have been horses, cattle, goats, monkeys, dogs, rabbits, guinea-pigs, white rats and mice. The inoculations were made, as a rule, intraperitoneally. Inoculation experiments with *Trypanosoma dimorphon* are also given for purposes of comparison. These are printed in italics:—

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.	Remarks.
Horses.				
Edington …………	Unknown	Unknown	Unknown	Dr. E. thinks ran sub-acute course
„ …………	Horse	7	„	Living after 18 days when Dr. E. left
Dutton and Todd	*Natural infection*	*Unknown*	*2·5 years, still alive*	*Blood still infective*
„	„	„	*1 year, still alive*	*No record*
Laveran …………	—	„	—	*Horse recovered*
Cattle.				
Edington …………	Horse	12	Unknown	Animal looked well on 21st day
Dutton and Todd	—	*10·5*	*30*	*Two cattle*
Goats.				
Edington …………	Horse	Unknown	Unknown	Blood never showed trypanosomes up to 18th day
Dutton and Todd	—	*3·5*	*Well after a year*	*Two goats*
Monkeys.				
174 ………………	Rat	3	22	Spleen enlarged. General glandular enlargement
175 ………………	„	3	—	Still alive (Sept. 11, 1908)
Thomas and Breinl	—	*4 and 6*	*160 and 75*	*Two monkeys*
Dutton and Todd	—	*4*	—	*Two never became infected*
Dogs.				
165 ………………	Rat	7	18	Spleen enormously enlarged
166 ………………	„	9	15	„ „
167 ………………	„	7	17	„ „
179 ………………	„	7	14	Marked ulceration of stomach
180 ………………	„	9	14	Spleen greatly enlarged
181 ………………	„	7	14	„ „
Thomas and Breinl	—	*4 to 8*	*10 to 19*	
Dutton and Todd	—	*8*	*29*	*Average of 4 days*
Rabbits.				
70 ………………	Rabbit	25	—	Still alive after 146 days
84 ………………	„	10	—	„ „ 136 „
118 ………………	„	12	100	Spleen 4 inches long and much thickened
119 ………………	„	15	—	Still alive after 103 days
120 ………………	„	12	19	Spleen enlarged
Thomas and Breinl	—	*9*	*Acute, 26—35; chronic, 78—157*	
Dutton and Todd	—	*13*	53	*One rabbit*

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.	Remarks.
		Guinea-pigs.		
82	Rabbit	25	134	Liver and spleen enlarged
110	Rat	12	43	" greatly enlarged
111	"	19	43	" "
124	"	21	—	Still alive after 105 days
125	"	21	69	Liver and spleen enlarged
126	"	41	65	" "
Dutton and Todd	—	*6*	*30*	*Two guinea-pigs*
Thomas and Breinl	—	*4 to 15*	*9 to 60*	
		White Rats.		
69	Rabbit	13	30	Typical *post-mortem* appearances
83	"	10	—	Killed for cultivation experiments
83A	"	11	—	" "
127	"	7	41	Usual *post-mortem* appearances
128	"	7	36	" "
88	Rat ii	5	20	2nd passage through rat
89	" ii	5	—	Killed for cultivation experiments
106	" ii	5	—	" "
107	" ii	4	14	Typical *post-mortem* appearances
108	" ii	7	35	" "
109	" ii	5	28	" "
102	" iii	6	—	Killed for cultivation experiments
102A	" iii	7	37	Usual *post-mortem* appearances
121	" iii	4	37	" "
122	" iii	5	44	" "
123	" iii	4	—	Rat lost
156	" iii	7	20	Usual *post-mortem* appearances
157	" iii	7	23	" "
Dutton and Todd	—	*7*	*36*	
Thomas and Breinl	—	*4 to 7*	*7 to 42*	
		Mice.		
132	Rat	10	24	
134	"	4	11	
137	"	10	11	
Dutton and Todd	—	*5*	*16*	
Thomas and Breinl	—	*2 to 5*	*16 to 23* *37 to 130*	

Conclusion.—The results of these inoculation experiments with Dr. Edington's trypanosome and *Trypanosoma dimorphon* show that they act on the various animals employed in a strikingly similar manner.

CULTIVATION OF DR. EDINGTON'S TRYPANOSOME, *Trypanosoma dimorphon*, AND *Trypanosoma congolense.*

In June, 1903, Novy and MacNeal first announced the successful cultivation of *Trypanosoma lewisi.* In the same year and in the following year they also succeeded in cultivating *Trypanosoma brucei* and *Trypanosoma evansi.*

These gentlemen deserve the highest possible credit for this most difficult achievement, an achievement which most workers in this subject thought impossible. The amount of work they expended and the splendid intelligence and pertinacity with which they pursued their object, refusing to accept defeat, command the admiration of all their co-workers in this branch of biological science. Since then the trypanosomes of birds, frogs, and fish have been cultivated by the same and other workers; but these successes have only been made possible, as a rule, by the pioneer work of Novy and his assistants. Coming out of their work, mention may also be made of the very interesting and important observation made by Rogers when he grew Leishman's bodies in ordinary citrated blood into trypanosome-like flagellates.

One of the chief interests attaching to this cultivation of trypanosomes is that it may assist in separating the different species of these organisms. At the present time trypanology is in a state of chaos on account of this difficulty in differentiation. Many diseases of animals caused by trypanosomes have been reported from all parts of Africa, Arabia, India, the Philippines, Mauritius, etc., and it has often been found impossible to name the species of trypanosoma causing them with any approach to certainty.

As mentioned above, the usual method of separating the different species is by taking into consideration the morphology, the result of inoculation into animals, the cross-immunisation methods and serum diagnosis of Laveran and Mesnil, the mode by which the disease spreads from the sick to the healthy—by a tsetse fly, a stomoxys, a tabanus, or by contact, as in dourine—by the effect of various drugs, cultivation, etc.; and, as already stated, the effect the parasite has on animals and the mode of conveyance are probably, for practical purposes, the most important. But to assist in separating the various species, cultivation has been of use in the past, and, as the methods become perfected, will be of still greater use in the future.

The following description of the cultural characters of Dr. Edington's trypansome exemplifies this, for, by comparing them with the cultural characters of other pathogenic species, a fairly shrewd guess at its classification may be made by this means alone. For the purpose of this comparison a compilation of the cultural characters of *Trypanosoma lewisi*, *Trypanosoma brucei*, and *Trypanosoma evansi* has been made from the writings of Novy, MacNeal, and Smedley.

It may be mentioned here that attempts have been made in this laboratory to cultivate these three species. The cultivation of the first was found to be a comparatively easy matter; but all attempts, and they were many, to cultivate the last two have, up to the present, failed, although Novy's instructions were carefully followed.

Cultivation Medium used.

The blood-agar medium used was made according to instructions kindly sent by Prof. Novy. These need not be repeated here, as the details are fully given by Novy and MacNeal in various papers.

CULTURAL CHARACTERS OF *Trypanosoma lewisi.*

A. *Living, unstained.*

Size.—Varies considerably in size. Some are not more than 1 or 2 microns long, not including the flagellum. Others are about the diameter of a red blood corpuscle, while the usual length of the spindle-shaped cells is 15 to 20 microns. Some trypanosomes can be found at times which are 50 to 60 microns long. The greatest variation in size is found in young cultures.

Shape.—*Trypanosoma lewisi* varies greatly in shape, as well as in size. Round, pear-shaped, fusiform and slender forms are present in the cultures. The round forms are usually found in old cultures, and are probably involution forms.

Contents of Cell.—The protoplasm in *Trypanosoma lewisi*, especially in young cultures, is bright, glistening, and apparently homogeneous in structure in the fusiform and slender forms.

Undulating Membrane.—Not present as far as can be seen. The movement of these cultural forms appears to be entirely due to the rapid motion of the flagellum.

Flagellum.—These forms possess, as a rule, a long free flagellum. In the slender forms this is sometimes twice the length of the body.

Motion.—The single, slender, cultural forms of *Trypanosoma lewisi* are very active, and dart across the field of the microscope in a straight line. In older cultures the round and other involution forms do not, as a rule, show more than a slight swaying movement.

Colonies or Aggregations.—Growth commences in a first generation about the fifth day by the appearance of small rosettes composed of a few trypanosomes. The colonies rapidly grow, so that on the following day masses of wriggling trypanosomes may be seen. These aggregations of twenty or more are attached by their flagella. They grow larger and larger until, about the twenty-fourth day, they are apparent to the naked eye, and consist of many thousands of trypanosomes.

B. *Fixed, stained.*

Protoplasm.—Homogeneous, as a rule. Vacuolation is rare, but sometimes a large highly-refractile vacuole is seen.

Nucleus.—Round or oval in shape. Situated centrally or at the junction of the anterior and middle thirds.

Micro-nucleus.—Is placed either close to the nucleus or at a variable distance anterior to it. In the free forms it is never seen lying posterior to the nucleus. As a rule, it is a rod-shaped structure, lying transversely to the long axis of the trypanosome.

Flagellum.—Arises from the vicinity of the micro-nucleus. The free flagellum is often two, three or four times the length of the body of the trypanosome.

Undulating Membrane.—In the cultural form of *Trypanosoma lewisi* this structure is apparently absent.

Colonies or Aggregations.—There is little to add to the description of the trypanosomes and of their arrangement in colonies. Stained preparations show that the trypanosomes sometimes possess very long flagella. Novy and MacNeal* have not apparently succeeded in staining the flagellum in their preparations, though they noted the position of the centrosome. They expressed the opinion that the end of the trypanosome pointing towards the periphery of the colony was the anterior extremity, and that from it a flagellum would arise if the cultural conditions were perfected (Smedley).

Measurements of the Cultural Forms of Trypanosoma lewisi.

Pear-shaped Forms.—(1) Body, 3·6 to 4·4 microns long, and nearly as broad. (2) Flagellum, two to four times the length of the body.

Spindle-shaped Forms.—14 to 16 × 2·4 to 3·5 microns, flagellum not included.

Smaller and larger forms are frequently found.

The adult parasitic form of *Trypanosoma lewisi* measures 24 to 25 × 1·5 microns (Laveran and Mesnil) (Smedley).

CULTURAL CHARACTERS OF *Trypanosoma brucei.*

A. *Living, unstained.*

Size.—Shows less variation in size than *Trypanosoma lewisi*, and averages 15 microns in the living condition. Smaller than those found in the blood.

Shape.—Do not vary much in shape, and closely resemble the forms found in the blood (Smedley).

Contents of Cell.—Show one or two very large, bright, and highly-refracting globules, usually placed near the anterior or flagellar end, in the otherwise homogeneous colourless cell. In size the globules may attain 1 micron

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* 'Cultivation of *Trypanosoma brucei*,' p. 28.

At times the number of these globules is increased, as when the culture is kept at 34° C. The presence of numerous large, highly-refractile globules in the cultural forms of *Trypanosoma brucei* is attributed by Novy and MacNeal to degeneration of the organisms, owing to imperfection of the culture medium. These globules become more numerous as the age of the culture advances. Do not seem to alter in position or shape if kept under observation for several hours. Resist staining completely. Laveran and Mesnil suggest that the globules are of the same nature as the refringent, unstainable granules found in *Trypanosoma rotatorium.*

Undulating Membrane.—No detailed description available.

Flagellum.—The flagellum in the living cell is by no means as distinct and as long as that of *Trypanosoma lewisi.*

Motion.—The motion of *Trypanosoma brucei* is slow and wriggling, and only exceptionally is a slowly-progressive form observed. The wave-motion slowly passes along the thick, undulating membrane, and gives the appearance of a spiral rotation to the entire cell. Scarcely departs from its place (Novy). In a young culture the trypanosomes are found to possess very active movements. Sometimes they advance across the field moderately quickly, but their rate of movement is always much slower than that of the rat trypanosomes, whose flagella are longer and more rapid in motion (Smedley).

Colonies or Aggregates.—Occurs in groups or rosettes. Rarely forms masses of more than 10 to 20 cells. The individuals are long, narrow, and show the peculiar writhing motion. The flagella are directed outwards, and the appearance of the whole may be compared to the snakes on a Medusa head. The stellate group with the bright, refracting globules within the cells, suggests a jeweller's "sun burst" (Novy). The active movements of the trypanosomes, and the large glistening vacuoles with which they are studded, give these colonies a singularly beautiful appearance (Smedley).

B. *Fixed, stained.*

Protoplasm.—The protoplasm invariably contains a few deeply-stained granules of a red or violet colour. The vacuoles are seen as clear circular spaces with sharply-defined outlines in stained preparations (Smedley).

Nucleus.—Round or oval in shape; and in older forms it breaks into masses of cromatin, which are found distributed through the protoplasm of the cell (Smedley).

Micro-nucleus.—This is much smaller than in *Trypanosoma lewisi;* it is usually circular, but sometimes elongated. It stains a deep red or purple colour, and it is sometimes difficult to distinguish it from the other granules.

It is generally found close to the vacuole; sometimes it lies close to the nucleus, but it is nearly always posterior to the latter structure (Smedley).

Flagellum.—Takes a tortuous course along the free border of the undulating membrane, and projects for a short distance from the anterior extremity (Smedley).

Undulating Membrane.—No detailed description given.

Colonies or Aggregates.—Most of the flagella are directed in an outward direction. It is rare to find colonies of a large size (Smedley).

Measurements of the Cultural Forms of Trypanosoma brucei.

Length, including flagellum, 18 to 23 × 2·5 to 3·5 microns. Length of free flagellum, 3 to 5 microns. Diameter of vacuoles, 1 to 2 microns. The adult parasitic forms of *Trypanosoma brucei* measure, in the blood of rats, 26 to 27 × 1·5 to 2·5 microns (Laveran and Mesnil) (Smedley).

CULTURAL CHARACTERS OF *Trypanosoma evansi.*

A. *Living, unstained.*

Size.—The body of one large individual measured 21 microns, while the flagellum was 28 microns in length.

Shape.—The slender fusiform body terminates at one end in a delicate flagellum. The posterior end, especially when blunt, showed a rod-like tip or stylet, which varied from 2 to 4 or even 6 microns in length. As the cultures aged, pear-shaped or spherical, highly granular, involution forms appeared. In the former type, measuring about 3 by 5 microns, the end was often provided with a flagellum, 10 to 15 microns long, which still showed a slow lashing movement, though the cell itself was motionless. The spherical forms varied from 4 to 9 microns in diameter, were granular, and often showed a remnant of the flagellum as a short, stiff, motionless whip. These involution forms, as in the case of *Trypanosoma lewisi* and *Trypanosoma brucei*, eventually gathered into large groups or masses, which at times filled the field of an immersion lens. Later on, the round bodies broke up into masses of very minute granules.

Contents of Cell.—Presence and peculiar arrangement of granules within the cells, and a distinct yellowish or greenish colour of the granules and of the contents. Large numbers of small granules or globules, which vary from 0·3 to 0·5 micron in diameter. These globules, as well as the contents of the cell, possess a decided yellowish or greenish colour, and appearance quite unlike that of either *Trypanosoma lewisi* or *Trypanosoma brucei*. The globules are usually massed in the anterior-third of the cells—that is, at the base of the flagellum, and only a few isolated granules are scattered through the remainder of the organism (Novy and MacNeal).

Undulating Membrane.—Is not recognisable in the living organism.

Flagellum.—Usually as long and often even longer than the cell itself.

Motion.—All single and actively motile, traversing the field of the microscope at great speed. Travel with the flagellum in rear or in front.

Colonies or Aggregates.—Entire absence of the groups or rosettes, which are so characteristic of the cultures of *Trypanosoma lewisi* and *Trypanosoma brucei*. The trypanosomes were all single and actively motile.

Measurement of the Cultural Forms.

Length, including flagellum, 25 to 50 by 1·5 to 2·5 microns.

CULTURAL CHARACTERS OF DR. EDINGTON'S TRYPANOSOME.

A. *Living, unstained.*

No difficulty is found in cultivating Dr. Edington's trypanosome. As early as the second day, if kept at 25° C., it is found to have greatly increased in numbers. The single individuals are in active motion, the flagellum wildly waving, while the body slowly moves among the corpuscles. Many dividing forms are seen with two or three flagella. Masses or aggregations are also seen varying in size, from those composed of a dozen individuals to those occupying a fifth of the field. These aggregation-forms are all writhing and squirming, while the flagella at the periphery are frantically waving. This incessantly moving mass, dotted over as it is with many small bright vacuoles, makes a curious and beautiful microscopic object when brightly illuminated.

On the third day the trypanosomes have multiplied to an extraordinary extent. Huge aggregations are now seen, each filling up several fields of the microscope. The individual trypanosomes are still actively motile. Single, double, and small aggregations are also seen.

By the seventh day they have reached the height of their growth and begin to degenerate.

After the twelfth day living forms can no longer be recognised in the culture tubes.

Size.—Dr. Edington's trypanosome, examined in the fresh living condition, varies considerably in size. Some of the large forms measure 32 microns in length, whereas the smaller are only half that length, or even shorter.

Shape.—So also in regard to shape, these cultural forms vary extremely. Round, oval, pear-shaped, and irregular forms are seen. Slender forms shaped like ordinary trypanosomes, with a beak or rostellum at one end, a fairly thick flagellum at the other, and furnished with an undulating membrane, are fairly common. Large irregular masses of any shape, furnished with one or more flagella, are also frequent.

Contents of Cell.—These cells have a remarkable appearance, as they are filled with highly refractile granules, large in size, round in shape, and numerous.

Undulating Membrane.—The round, oval, and pear-shaped forms do not appear to possess an undulating membrane, whereas the long, slender forms, as also the huge fish-shaped or octopus-like forms, often show well-marked undulating membranes.

Flagellum.—The flagella in these living unstained cultural forms are thick and coarse, and differ markedly from the slender structures usually associated in the mind with trypanosomes. Just as in the parasitic forms found in the blood, it is evident that the protoplasm of the body extends to the tip of the flagellum giving rise to this thick stumpy appearance.

Motion.—The slender forms are active and swim fairly quickly across the field. The large, irregular forms are stationary, but exhibit actively wriggling flagella and amœboid movements of the body substance.

Colonies or Aggregates.—Colonies or aggregations of 10 to 20 individual cells are common. The cells are arranged irregularly. Some of their flagella may be directed outwards, while others are seen entangled in the mass and feebly wriggling. On the third day these aggregations may be seen as large as three to five fields of the microscope, and must be composed of many thousands of individual trypanosomes.

B. *Fixed, stained.*

Method of Fixing and Staining.—The cultural forms of Dr. Edington's trypanosome were either prepared by mixing a drop of the cultivation fluid with fresh serum, spreading on a slide, and staining by Leishman's modification of Romanowsky's method, or the fluid was spread on a slide, fixed by osmic acid and stained by Giemsa, and then treated with orange tannin to differentiate the various structures.

In Leishman-stained preparations the protoplasm of the cells is stained a pale blue, the nuclei and irregular masses of chromatin reddish or pink, while the vacuoles stand out as unstained spaces with sharply-defined margins. In Giemsa-stained preparations, on the other hand, the protoplasm is stained a pale puce colour, while the chromatin material is stained reddish purple.

Protoplasm.—The protoplasm of the cell is homogeneous, but contains irregular-shaped granules and masses of chromatin-staining material. There are also present numerous well-marked vacuoles of various sizes, which are unstained, and, as mentioned above, highly refractile.

Nucleus.—The nuclei are of every form and shape, and often broken up into irregular masses.

Micro-nucleus.—The micro-nuclei are irregularly placed; in some cells are not easily distinguishable from other granules contained in the protoplasm, but in many are clearly seen as deeply-staining bodies, round or rod-shaped, in close connection with the point of origin of the flagella.

Flagellum.—The flagella are, as a rule, thick and fleshy. In the irregular forms they appear to spring from any part of the shapeless mass of protoplasm, and in any direction.

Undulating Membrane.—The undulating membrane is also characterised by its extreme irregularity. In many cells it appears to be absent, while in others it is well marked, broad, and thrown into folds.

Colonies or Aggregations.—The individual trypanosomes which go to compose the large aggregations are as a rule short and stumpy in form, with oval-shaped nucleus and short stumpy flagellum. They are of irregular shape and size, and are placed without any seeming order.

CULTURAL CHARACTERS OF *Trypanosoma dimorphon* (DUTTON AND TODD).

It is unnecessary to describe in detail the cultural characters of this trypanosome, as they agree exactly with those of Dr. Edington's.

CULTURAL CHARACTERS OF *Trypanosoma congolense* (BRODEN).

Several attempts were made to cultivate *Trypanosoma congolense*, but none of them were very successful. There is certainly not the rapid growth of this trypanosome which distinguishes Dr. Edington's trypanosome and *Trypanosoma dimorphon.* It is only after a long search that individual trypanosomes can be found in the preparations. There is no formation of masses or aggregations filling several fields of the microscope as in the others. It is difficult to say whether there is any real multiplication or not. All that can be said is that, for about eight days, living trypanosomes can be seen. At first these are shaped like the ordinary trypanosomes found in the blood, only larger and swollen in appearance; but by the fifth and following days these change into most irregular and fantastic shapes. Nothing living could be seen after the eighth day. This cultivation experiment would therefore seem to strengthen Dr. Laveran's opinion that *Trypanosoma dimorphon* and *Trypanosoma congolense* are distinct species.

Conclusion.

The conclusion arrived at is that Dr. Edington's trypanosome from Zanzibar is probably Dutton and Todd's *Trypanosoma dimorphon.* One link in the chain of evidence, however, is wanting, and that an important one—the identity or non-identity of the carrier.

DESCRIPTION OF PLATES.

PLATE 1.

This plate represents the shape and size of the three Trypanosomes, viz. :—

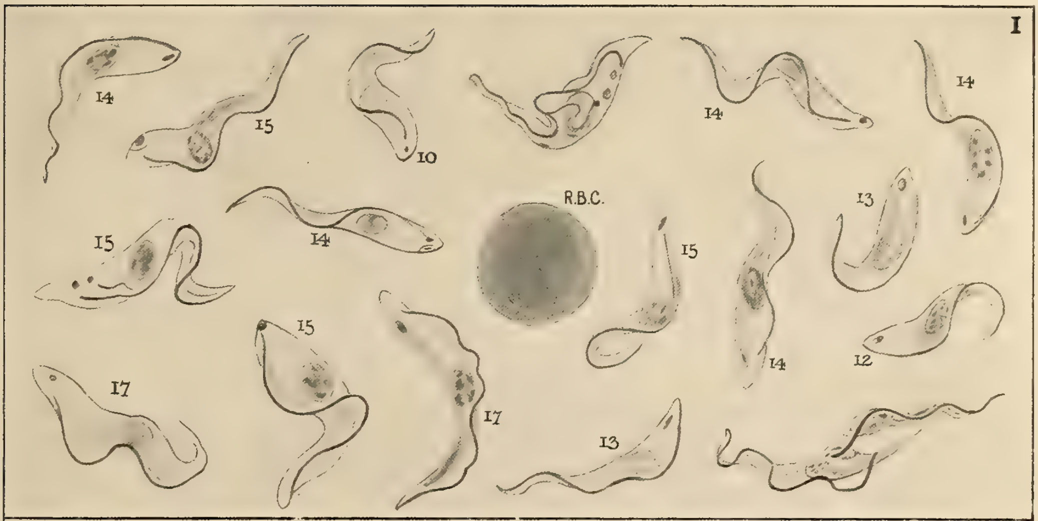
1. Dr. Edington's trypanosome. From blood of rat. 30th day of disease. Stained Giemsa. × 2000. See p. 27.
2. *Trypanosoma dimorphon.* From blood of rat. 15th day of disease. Stained Giemsa. × 2000. See p. 29.
3. *Trypanosoma congolense.* From blood of mouse. 7th day of disease. Stained Giemsa. × 2000. See p. 29.

PLATE 2.

FIG. 1.—Part of an aggregation of Dr. Edington's trypanosomes after 5 days' growth. Stained Giemsa. × 2000.

FIGS. 2–4.—Dr. Edington's trypanosome after 6 days' growth. Stained Giemsa. × 2000.

FIGS. 5–14.—Cultural forms of Dr. Edington's trypanosome after 7 days' growth. Stained Leishman. × 2000.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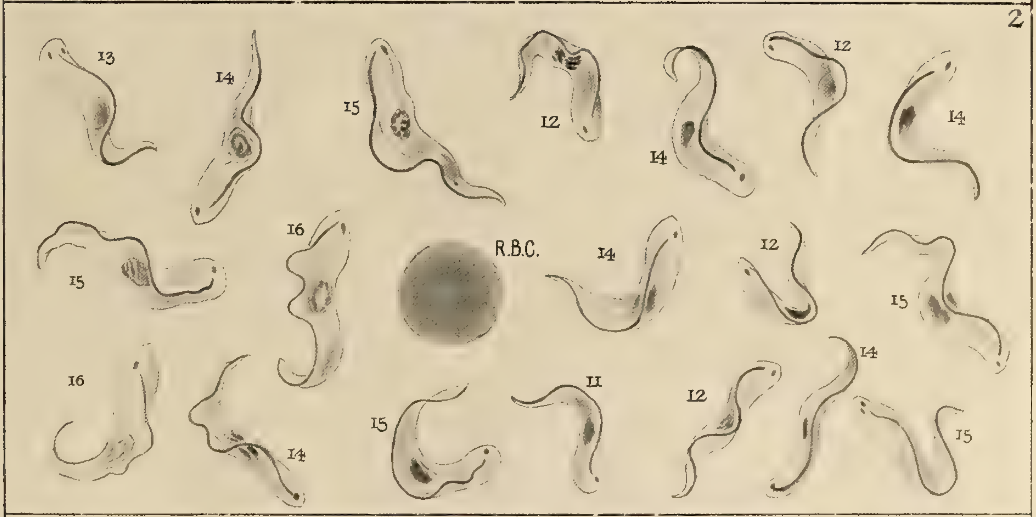
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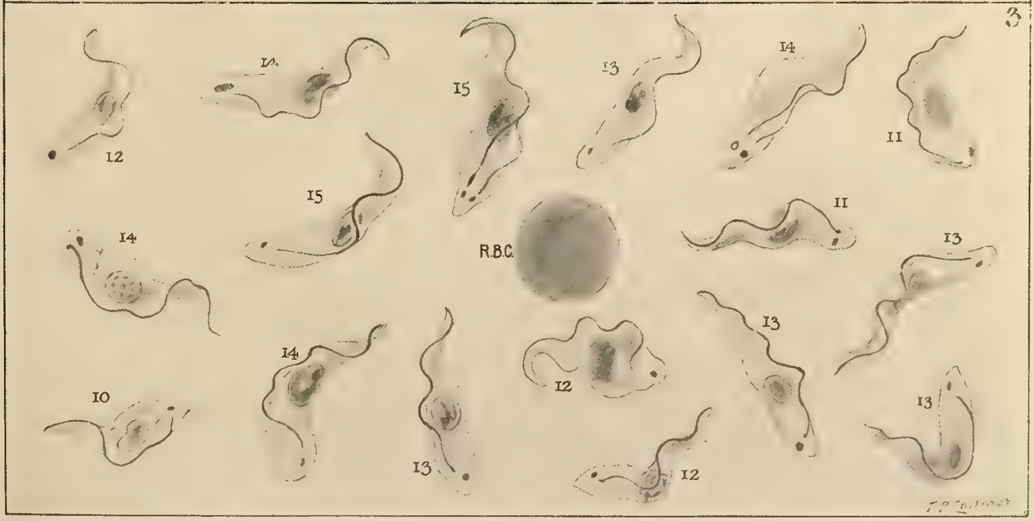
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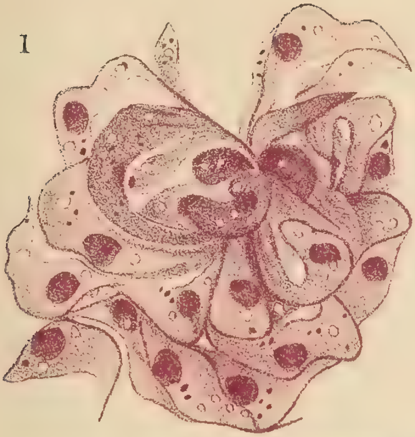

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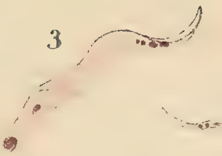

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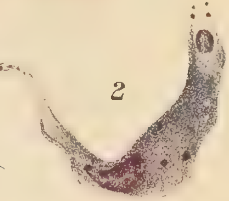

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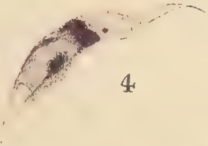

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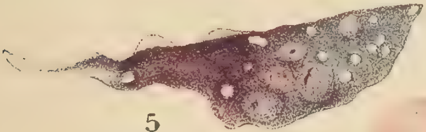

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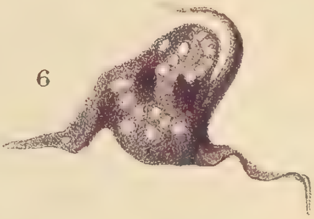

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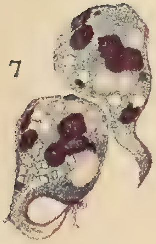

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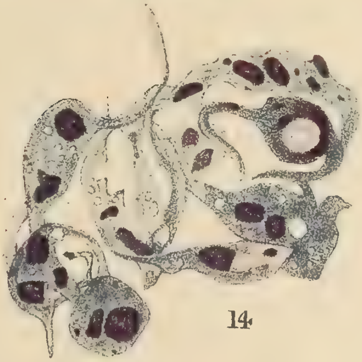

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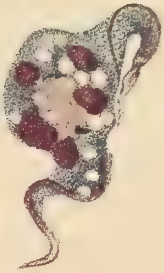

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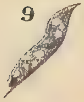

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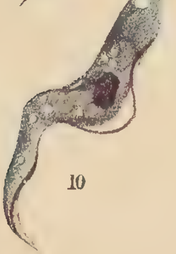

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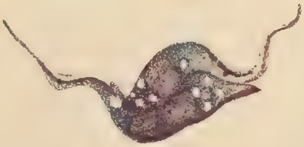

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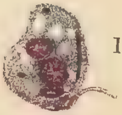

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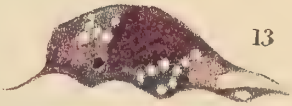

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A Summary of further Researches on the Etiology of Endemic Goitre.

By Robert McCarrison, M.B., B.Ch., Captain, Indian Medical Service.

(Communicated by Major Ronald Ross, C.B., F.R.S. Received October 24,—
Read November 26, 1908.)

The object of the research was to determine by experiment on man whether goitre was caused by matter held in suspension in goitre-producing waters; and to ascertain, as far as possible, the nature of the suspended ingredient which had been surmised to be responsible for the production of the disease.

Thirteen individuals, including myself, were given suspended matter, which had been removed by filtration from goitre-producing water, every morning before the first meal of the day. I and three others developed enlargements of the thyroid gland. The experiment was repeated in the case of eight individuals who were given the same suspended matter, which had previously been boiled for 10 minutes; in no case did any enlargement of the thyroid gland occur.

It is concluded from these results that goitre is due to a living organism of disease present in the water. The incubation period of experimentally-produced goitre was 13 to 15 days.

It is thought probable that the organism of goitre exists as an intestinal parasite in goitrous individuals, since an intestinal antiseptic appeared to have a marked curative effect.

Experiments were made on monkeys to test the possibility of the spread of the disease by the fæces of infected individuals, with negative results.

Plentiful amœbic infection of the intestine was found in the majority of cases of goitre examined. It is not known, however, whether amœbæ have any relationship to the disease.

The research was carried out in Gilgit (Kashmir), and the results obtained refer only to goitre as it occurs there.

The Proportion of the Sexes produced by Whites and Coloured Peoples in Cuba.

By Walter Heape, M.A., F.R.S., Trinity College, Cambridge.

(Received September 30,—Read November 26, 1908.)

(Abstract.)

Introduction.—Darwin, in his great work on the Descent of Man, deals with the proportion of the sexes in various animals and the power of natural selection to regulate the proportional number of the sexes. He recognises a general tendency to equality of the sexes but remarks on the fact that this equality is often greatly disturbed. In certain rare cases of marked inequality he concludes they might have been acquired through natural selection, but in all ordinary cases, such as, for instance, the difference in the proportion of the sexes in legitimate and illegitimate children, it can hardly be so accounted for and must be attributed to unknown conditions, although, he adds, natural selection will always tend to equalise the relative number of the two sexes.

About that time a host of writers were engaged in investigating various possible causes for this inequality, and many theories were promulgated to account for it, such as the relative age of the parents, the time of conception, and so forth. Prominent amongst them was Düsing, who set himself to show that nutriment was the chief determining factor. He set forth his case with great ability and brought an enormous mass of evidence in support of his view.

Students of heredity in those days claimed that the laws of heredity were sufficient to account for all inequalities, but Düsing emphatically denied this, and in my opinion satisfactorily showed he had sound reason for doing so.

During the last few years much work has been done on sex, especially regarding the factors which determine sex, and strong evidence has been brought to show that both individual spermatozoa and ova are themselves of definite sexuality. It is suggested that the sex of the individual resulting from the conjugation of a spermatozoan and an ovum must be determined by one or other of them, not by both, and it is claimed that, in order to fulfil the conditions, a M. ovum must be fertilised by a F. spermatozoan and, *vice versâ*, a F. ovum by a M. spermatozoan. So far as the evidence available now goes, it would seem possible that in some animals the sex of the offspring is determined by the spermatozoan and in other animals by the ovum. The facts are not clear, however, though it is to be hoped the efforts now being made by the Mendelians will make it so; at present, perhaps, all that can be said regarding mammals is that the evidence available is in favour of the view that the ovum determines the sex of the offspring in these animals.

Now a female mammal produces only a limited number of her ovarian ova during her life, others degenerate and are absorbed, in fact, some ovarian ova survive at the expense of others and it would appear that this process goes on with more or less activity at different times. Thus there is a struggle for existence and a process of selection going on in the mammalian ovary, and this is a very important fact, for the projection into the ovary itself of forces which are undeniably produced by extraneous conditions shows that such conditions must to some extent influence the output of the ovary.

A wealth of evidence has been adduced by many observers to show that M. and F. larvæ are very differently affected by different foods and different climatic conditions, and this evidence is overwhelmingly in favour of the view that F. larvæ require more nourishment, more favourable conditions, than do M. larvæ for their development. But if this is true for larvæ it is surely true also for ovarian ova, and the conclusion may be confidently drawn, that the selection of M. or F. ovarian ova, for production, is liable to be influenced by the food supplied to the ovary by the mother and therefore by the conditions of metabolic activity she experiences.

As all breeders know, the breeding power of an animal is in direct relation to its metabolic activity, and the metabolic activity of a mother is undoubtedly affected by the food supplied and the climatic conditions she experiences; thus it would appear that extraneous conditions must exert influence on the proportion of the sexes produced by all animals in which a struggle for existence takes place among the ova in her ovary.

This view, it appears to me, explains a variety of facts which have been judged to be contradictory, and brings into line the results of many observations which have hitherto been supposed to favour now one, now another, quite different theory. For instance, all the contradictory evidence I have examined regarding the effect on the sex ratio of the ages of the parents and the times of conception, may be so accounted for, while upon the phenomena concerning sex ratio observed in consequence of crossing or of in-breeding, a new light is thrown which will, I think, go far to show adequate reason for the results obtained for mammals. It must not be supposed that I attribute the proportion of the sexes produced to these agencies alone; there can be no doubt, in my opinion, that heredity is the main force at work, but it is incontrovertible that variations in that proportion constantly occur, and I maintain that these variations cannot be accounted for by any law of heredity and are referable to those extraneous forces which act as selective agents on the ovarian ova. The evidence I have to offer in the following paper is, I think, strongly confirmatory of this view.

1. *Data dealt with.*—From a statistical point of view, human beings are the only mammals for which sufficiently large numbers can be obtained with any hope of ensuring sufficient accuracy, and for these there has always been difficulty in assuring oneself of the completeness of the records at any one time for more than one race. When, therefore, my friend, Dr. F. H. H. Guillemard, pointed out to me that the publications of the chief sanitary officer of Cuba supplied separate details of the births and still-births of whites and coloured people in the island, and that these records were further subdivided into legitimate and illegitimate births and still-births, I communicated with that officer (Dr. Finlay), and he has very kindly supplied me with a complete series of his monthly publications for the years 1904–5–6. It is with these records I now deal. The numbers dealt with amount to—

Births—whites, 131,721; coloured, 39,576. Total, 171,297.
Still-births—whites, 4160; coloured, 2247. Total, 6407.
Total production—whites, 135,881; coloured, 41,823. Total, 177,704.
Deaths—whites, 52,087; coloured, 27,877. Total, 79,964.
Marriages—whites, M. 31,481, F. 31,240; coloured, M. 7598, F. 7839. Total, M. and F. (each) 39,079.

These totals are arrived at from monthly records, for each of these three years, for each of the six provinces into which the island is divided; and in each case, except for marriages, the proportion of M. per 100 F. has been calculated. Altogether I have drawn up 64 tables of these and similar details; they are dealt with more fully elsewhere, the results thus obtained I now summarise.

2. *The Racial Proportion of the Sexes.*—The first prominent fact demonstrated is the difference in the proportion of the sexes produced by the two races. The whites produce a larger proportion of M. than the coloured people. For whites, the proportion varies during these three years from 106·8 to 110·52, in the total it is 108·44 M. per 100 F.; for coloured, the proportion varies from 101 to 101·2, total 101·12 M. per 100 F.; and this racial difference is shown both for births and still-births.

Here, then, we have a marked racial difference which, after examination of other statistics of coloured people and of the inhabitants of Spain (from whence most of the white inhabitants of Cuba originally came), I am of opinion may confidently be assumed to show that, in this particular, the influence of heredity is clearly demonstrated.

3. *The Sexual Ratio in Legitimate and Illegitimate Births.*—The second

prominent fact is the consistent variation in the proportion of the sexes produced in consequence of legitimate or illegitimate union. This is evident both for births and still-births in both races, and emphatically shows that illegitimate union results in the production of a marked increased proportion of F. For whites, the total legitimate births show 107·78, while the illegitimate show only 104·4 M. per 100 F. For coloured, legitimate births show 106·76, and illegitimate 96·76 M. per 100 F. The records of still-births show a difference of 10·06 more M. among legitimate still-births for whites, and 16·63 more M. in that class for coloured people; but the proportion of M. among still-born children is vastly higher than among births, the totals for whites and coloured for the three years being in proportion of 144·45 M. per 100 F.; thus, when births and still-births are added together, the result of legitimate union among whites gives 109, of illegitimate union 105·95 M. per 100 F.; among coloured, legitimate unions give 107·73, and illegitimate 97·91 M. per 100 F. Thus this difference, while it is much greater for coloured than for white people, is marked for both races in the totals, and is shown to be a remarkably consistent variation throughout my tables.

It is clear that illegitimate union amongst civilised peoples is due to individual characteristics in the woman which have for their basis a specially active sexuality. Thus the result of illegitimate union, the increased production of F. in consequence thereof, is an individual matter, it cannot be accounted for by any law of heredity and must be associated with physiological conditions which induce this special activity, that is to say with forces which affect the metabolic activity of the woman. I cannot here detail all the arguments in favour of this view and will only add (the evidence admits, I think, of no other interpretation) that, as I have already shown, an exceptionally active metabolism in the mother should favourably affect the development and ripening of F. ova, and that this is what is found to be the case here.

4. *Breeding Seasons.*—An examination of the monthly tables demonstrates the existence of two sharply-defined breeding seasons each year, and shows that they are experienced by both whites and coloured at the same time. One breeding season is more marked than the other, this fact is also common to both races, but both are quite unmistakably shown in the birth tables. The records of marriages show that the marriage season, though it is also quite definitely indicated, has no relation whatever to the breeding seasons. On the other hand, reference to records of temperature, barometric pressure, humidity, etc., shows that these bursts of reproductive activity always take place at times when there is a marked change of climate; the one in the

autumn shortly after a sudden change from great heat to cooler weather, the other in the early months of the year at a time when the cool winter weather gives place to spring. The increased reproductive activity of the people is suddenly acquired and almost as abruptly allayed, it is obviously not a definite temperature but the experience of *a change of temperature* which induces this boisterous generative activity.

The same conditions are found to influence, in a similar manner, other animals which experience breeding seasons, and the effect on stock is increased metabolic activity. There can be no doubt these breeding seasons of the two races in Cuba are brought about by forces which tend to greatly increase the metabolic activity of the individual.

5. *The Effect of the Breeding Seasons on the Proportion of the Sexes produced.*—If, then, I am right in stating that the breeding seasons are induced in consequence of increased metabolic activity, and if my reasoning is sound regarding the increased output of F. among illegitimate births and the influence of different degrees of metabolism on the ripening and production of ovarian ova of different sexes, the result of the breeding seasons should show this.

It does show it, emphatically; my tables demonstrate that the greatest excess of F. is produced at times of greatest fertility, *i.e.* during the breeding seasons, when the metabolism of the mother is most active. This is true for both races and it is clearly shown in all totals and in the totals for each individual month, except for two months of one year for coloured people.

I feel convinced such a variation in the sex ratio cannot be ascribed to the action of any law of heredity; it is clearly associated with the exercise of extraneous forces on the ovary and is, I submit, due to those forces.

6. *The Limitation of the Influence of Extraneous Forces.*—In connection with the above, another fact is shown which is of considerable interest, namely, that while whites show a more marked sensibility to the influences which induce the production of F., coloured people are more affected by the forces which stimulate the production of M., and this condition is more marked among illegitimate than among legitimate birth records.

This fact shows that the race which normally produces a considerable excess of M. is most amenable to the forces which induce the ripening of F. ova, while the race which produces the greatest proportion of F. reacts more generously to the influences which favour the production of M. ova. In other words, there is here demonstrated the exercise of a force which limits the power to produce an excess of either sex, a force which makes for some point near equality of the sexes, and which is, I take it, the force of heredity.

This exemplifies the nature of the claim I made; extraneous forces undoubtedly exist which effect a variation in the sex ratio, but they are to some extent subordinate to laws of heredity; nevertheless these former forces cannot be ignored, they are certain to interfere to some extent with the performance of the laws of heredity and with all calculations regarding sex ratio which are based solely upon those laws.

7. *The Effect of Town as compared with Country Life on the Sex Ratio.*—Finally, on analysis, my figures show another fact, namely, that a quite considerably higher proportion of F. are born in towns than in the country districts. This is shown in my tables for both races and is evident as a rule in the records for both legitimate and illegitimate births.

I have elsewhere discussed the reason for this; it is quite clear no law of heredity can explain such a variation, and I have concluded that the extraneous forces which are accountable for it must again be associated with the degree of metabolic activity experienced by the mother under variable conditions.

8. *Conclusion.*—Other facts of considerable interest in relation to this work are set forth elsewhere and I will not refer to them here. I have given above three instances of conditions under which the production of M. and F. children shows a marked variation from the normal. The results are similar for both the whites and the coloured races in Cuba. These people have hereditary qualifications which, in the main, govern the proportion of the sexes they produce, but conditions undoubtedly occur under the influence of which that proportion is varied. This variation is similar in character but different in degree for the two races, and is directly associated with definite extraneous forces, food and climate, which affect the metabolic activity of the mother.

Taken singly any one of these instances might be thought to be inconclusive, but taken together they seem to me to present strong evidence of the truth of my contention, that the variable metabolic activity of the mother, acting upon the ovary, induces a struggle for existence between the ovarian ova of different sexes, and affects the proportion of M. or F. ova which ripen and which are produced for fertilisation.

It is worthy of notice that these same extraneous forces must affect the proportionate produrtion of individuals possessing various kinds of different characters (quite other than sex) which are associated with metabolism, and, when better understood, may have valuable bearing on the means for selection of healthy ova and for preventing the maturation of ova bearing the active germs of disease.

Electrolytes and Colloids.—The Physical State of Gluten.

By Prof. T. B. Wood and W. B. Hardy, F.R.S.

(Received October 24,—Read December 10, 1908.)

Gluten, as ordinarily prepared by washing wheat flour in tap water, forms a coherent stringy mass insoluble in water. It consists essentially of a mixture of two proteins, gliadin and glutenin, but even when very thoroughly washed it always includes some starch. Gliadin, which forms rather more than half of the total protein, is soluble in dilute alcohol, and gives to the gluten its peculiar physical properties.

The power which dough possesses of retaining the gas formed during fermentation is due to the tenacity and ductility of gluten.* Therefore, the property of forming a light and well-shaped loaf, which is so variable a feature of different flours, is determined by the amount and the physical state of the contained gluten.

The physical state of gluten, like that of other colloids, is conditioned by the electrolytes which are present. Gluten washed out of flour with distilled water obviously is more friable and less tenacious than gluten washed out with tap water which contains salts. It is this influence of electrolytes upon the physical state of gluten which we propose to discuss.

Gluten is peculiarly sensitive to low concentrations of acid or alkali. A tenacious ductile mass suspended in a large volume of, for instance, 0·0001 normal acid, begins almost at once to show signs of disintegration, and is at once dispersed by slight movement to form a stable opaque colloidal solution or hydrosol.

Action of Acids.—This action was investigated quantitatively by suspending a small mass of gluten on a bent glass rod in a beaker containing 120 c.c. of a solution of acid of known strength, and noting the concentration at which cohesion was so far reduced as to allow the protein to fall off the rod and disperse in a cloudy "solution." It was found that while very dilute acid causes dispersion, a solution of a strong acid above a certain concentration maintains the cohesion. Gluten, therefore, is coherent in distilled water, and in strong acids above a certain critical concentration. A weak acid, such as acetic acid, brings about dispersion up to as high as twice normal, the highest concentration tried. Inspection of a series of beakers with concentrations of any strong acid from zero to the critical point makes it clear that, starting from the lowest concentration, dispersion increases to a maximum and then falls to zero at the critical point. In other words, the power of destroying the cohesion and dispersing the gluten as a cloud varies with the concentration of the acid, so that the relation can be shown by a curve. The form of the curve will be seen later.

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* Wood, 'Journn. Agric. Sci.,' vol. 2, part 2, p. 139, and part 3, p. 267.

The dispersion of the gluten is not due to a change in the protein molecule of the nature, for instance, of hydrolysis, since it can be recovered as a tenacious stringy precipitate by neutralising the acid or by the addition of salt.

The following table gives the mean of several determinations of the concentration at which the gluten retains its coherence. The exact point is the concentration at which gluten just breaks under its own weight when suspended in the solution of acid; and the results obtained in different experiments are fairly consistent. It is remarkable that there should be no simple relation between the observed concentrations and the strengths of acid used as measured by electric conductivity. The conductivity of the solutions after the gluten had been immersed in them was measured, and the results are given in the second column of figures, the value of the sulphuric acid solution being taken as unity:—

Table I.

Acid.	Normality of critical concentration.	Relative conductivity.
H_2SO_4	0·017	1·0
Camphorsulphonic	0·02	1·59
HNO_3	0·03	1·9
HCl	0·05	3·8
Oxalic	0·15	3·8
H_3PO_4	2·00	—

Action of Distilled Water.—Gluten breaks up when washed very thoroughly in many changes of ordinary distilled water. The distilled water used was acid to litmus owing to the presence of carbonic acid; and the dispersion of the protein is due to this acidity, since (1) it is precipitated by the addition of a trace of alkali, and (2) the protein when dispersed is electro-positively charged—that is to say, it displays the characteristic relation of protein to acid.

The Influence of Salts.—Salt in small concentration precipitates a hydrosol of gluten whether it be formed by acid or by alkali. Therefore, salts lessen the power which acids or alkalis possess of destroying the cohesion of gluten, and, in sufficient concentration, completely neutralise it. The concentration of salt necessary completely to nullify the dispersive power of particular acids was investigated in the manner already described, namely, by suspending approximately equal pieces of gluten in varying concentrations of acid and salt, and noting the point at which cohesion was so far reduced as to allow the protein to flow off the rod. The relations appear in the following curves (fig. 1), which show that for all strong acids and for all salts the concentration of the latter needed to balance the former increases to a maximum as the concentration of acid increases, and then declines to zero at the point where the acid alone is sufficient to maintain cohesion. The curves all agree, therefore, in showing that, measured by the concentration of salt needed to prevent dispersion, the dispersive power of an acid increases with increasing concentration, and then falls until the critical concentration is reached, where dispersive action is *nil.*

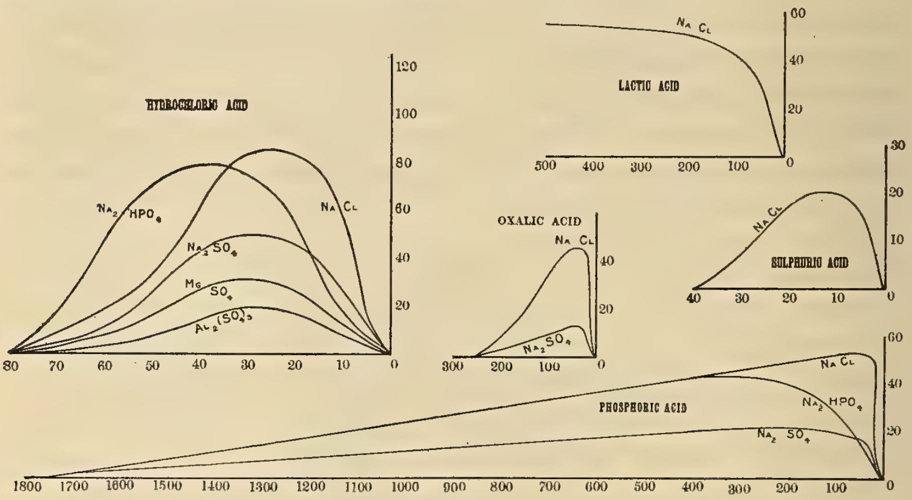

Fig. 1.

These curves are so characteristic that they afford a means of testing a point of general theoretical interest. One great class of colloidal solutions, the aqueous solutions of characteristically insoluble bodies such as metals, some proteins, sulphides, and gums, are characterised by the fact that round each particle of the solute there is an electric double layer, and on the potential difference between which the stability of the solution depends. Coagulation or precipitation of such a solution is approximately coincident with the reduction of the potential difference to zero, the most complete coagulation, *i.e.*, mechanically the densest and most coherent coagulum, being formed at the isoelectric point.*

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* Hardy, 'Roy. Soc. Proc.,' vol. 66, p. 110, 1900; Picton and Linder, 'Chem. Soc. Trans.,' 1905—1906, vol. 87; Perrin, 'Journ. de Chim. Physique,' vol. 2, p. 601, 1904; vol. 3, p. 50, 1905.

On this view the formation of the hydrosol of gluten is due to the development of electric charges round the particles of the protein owing to chemical interaction between the protein, the acid or alkali and the water; and the tenacity, ductility, and water-content of a solid mass of moist gluten depends upon the total or partial disappearance of these electric double layers, and the reappearance of what is otherwise obscured by them, namely, the adhesion or "idio attraction," as Graham called it, of the colloid particles for each other, which makes them cohere when they come together.

It is possible to put this hypothesis to the proof. We can measure the potential difference between the water face and the protein face of each particle in the hydrosol of gluten by determining the rate of transport of the particles in a uniform electric field. The method adopted has been described by one of us.* Briefly it consists in the use of a graduated U-tube, the opalescent hydrosol is introduced as the lower layer, the upper layer in each limb being a clear solution of the same electrical resistance. Electrodes are immersed in the upper layer, a field established, and the rate of movement of the boundaries between upper and lower layers observed.

The hydrosol was prepared either by washing gluten in distilled water containing carbonic acid, a process which occupied at least two days, or in a few hours by washing in a few changes of 0·0001 normal sulphuric acid. It was freed from all starch by centrifuging. To successive lots of the hydrosol, acid was added in varying amounts, and water when necessary, so that the concentration of protein was constant, while the concentration of acid varied. Finally the resistance was measured, and a fluid to form the upper layer was prepared either by adding the same acid to water or by adding sodium chloride. Hydrochloric, sulphuric, and acetic acids were used, and the results were in all cases the same. The figures for hydrochloric acid are plotted in the following curve, the ordinates being specific conductivity of the solution $\times 10^{-6}$, the abscissæ the specific velocity in centimetres per second for unit potential gradient $\times 10^{-}$ (fig. 2).

The curve agrees in form with those already given for the effect of salt upon cohesion, and we may therefore conclude that acids, and by inference alkalis also, destroy the cohesion of gluten by forming double electric layers round the particles, and that the potential difference between these layers rises with increasing concentration of acid to a maximum, and then falls.

Action of Alkalis.—The action of alkali in destroying the cohesion of gluten is essentially similar to that of acid, except that the electric sign is reversed. In a hydrosol of gluten formed by carbonic acid or any other acid the protein is charged positively; when formed by any alkali it is charged negatively.

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* Hardy, 'Journ. Physiol.,' vol. 33, p. 251, 1905.

It is interesting to note that, when alkali is added, it not only neutralises any acid present, but also reacts directly with the protein as though the latter were itself an acid. The alkali, therefore, disappears as such; it is, in point of fact, neutralised by the protein with the formation of new ions. For instance, in a particular hydrosol formed by carbonic acid, the particles of gluten were charged positively, and had a specific velocity 46×10^{-6} cm. per second. Sodium hydroxide was added in quantity sufficient to make the entire solution contain N/1600 of NaOH. The fluid was not alkaline to phenolphthalein, but in spite of this the protein was now charged negatively and had a specific velocity of 23×10^{-6} cm. per second.

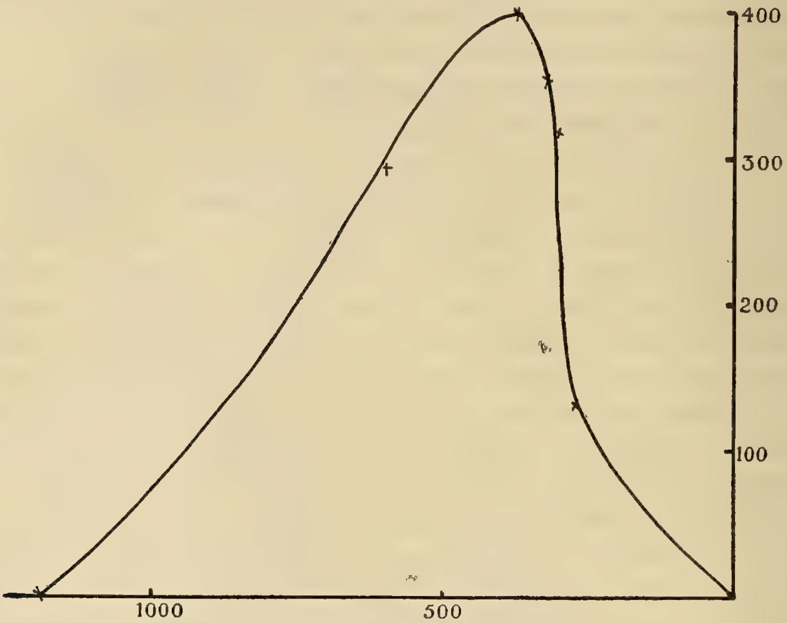

FIG. 2.

Approximately pure gliadin, dissolved in 70-per-cent. alcohol, shows relations to acid and alkali the same as those described for gluten. Dropped into 98-per-cent. alcohol or distilled water, it forms an opalescence, and is then electro-positive; in presence of N/4160 of NaOH it is electro-negative.

Conclusions.—The experimental results seem to prove beyond question that the physical state of gluten—that is to say, the degree of coherence or dispersion as a hydrosol, is determined by the potential difference between the particles of the protein and the fluid.

The development of such a potential difference between colloid particles and fluid has been accounted for in two ways. The first, which may be described as a purely physical hypothesis, ascribes it to differences in the speed of the ions of electrolytes present. The colloid particles at any moment contain within themselves an excess of the most penetrating and rapidly moving ions present, and they therefore have the charge of that ion. In presence of acid they will have the charge of the hydrogen ion, in the presence of alkali that of the hydroxyl ion. This hypothesis was advanced by one of us to explain the properties of certain proteins of the globulin class when in solution.* It was also advanced independently by Perrin to explain the electrical properties of colloidal solutions in general.†

The second hypothesis is frankly chemical in nature, and, as applied to proteins, it may be put as follows:—The protein molecule contains H and OH groups. Proteins, therefore, as a class are, like their chemical allies the amino-acids, amphoteric electrolytes. They react with acids and alkalis to form salts, but the reactions are not precise, an indefinite number of salts of the form $(B)_n BHA$ being formed where the value of n is determined by conditions of temperature, and concentration, and of inertia due to electrification of internal surfaces within the solution.

The salt so formed is ionised by the water. Positive or negative ions, as the case may be, leave the protein face to enter the water face, and form an electric layer there, while the protein face is left charged respectively negative or positive.‡ On this view, in the particular case under consideration, the decrease and final disappearance of the potential difference which occurs when the concentration of acid rises above a certain value would be due to a suppression of the feeble ionisation by the excess of acid.

The first view seems to be incompatible with certain experimental facts—such, for instance, as the fact that salts such as LiCl or LiBr, the velocities of whose ions are in the ratio of about 1 to 2, do not confer any change on proteins, nor, as Perrin noticed, do they produce any contact difference of potential between a water and a solid wetted by it. It also ignores the purely chemical nature of the conditions which govern the formation of colloidal solutions of metals.§

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* Hardy, 'Journ. Physiol.,' vol. 29, p. xxvii, 1903.

† Perrin, 'Journ. de Chim. Physique,' vol. 2, p. 601, 1904; vol. 3, p. 50, 1905.

‡ Hardy, 'Journ. Physiol.,' vol. 33, p. 251, 1905; 'Roy. Soc. Proc.,' vol. 79, p. 413, 1907.

§ Burton, 'Phil. Mag.,' vol. 11, p. 425, 1906.

The Colours and Pigments of Flowers with Special Reference to Genetics.

By M. WHELDALE.

(Communicated by W. Bateson, F.R.S. Received October 31, 1908,—Read January 28, 1909.)

Investigations, of which the following is some account, have been undertaken with a view to being of assistance in the interpretation of the phenomena observed in the inheritance of flower-colour. An attempt has been made to classify, of necessity roughly, the pigments, more especially those soluble in water, found in flowering plants, and at the same time to ascertain whether there is any connection between the genetic behaviour of pigments and their chemical reactions and constitution. On the basis of this classification it was thought that, at some future time, further investigations might be carried out in greater detail among the various classes of pigments.

This account deals more with yellow pigments than with red; attention was first directed to the yellow group, because of a certain correlation between reds and yellows observed in the inheritance of flower-colour in *Antirrhinum majus*, the relationship suggesting that a greater knowledge of yellows might be useful in classifying the reds. No detailed examination has been made of any one pigment, but merely a general survey of the colouring matters of genera from various natural orders.

A classification in outline of the pigments, other than chlorophyll, found in flowering plants has been given by many authorities,* but perhaps a repetition will not be out of place here, as follows:—

A. Pigments in solution in the cell-sap.†

(1) Soluble red-purple-blue pigments known as "*anthocyanin*"; that this term includes several different classes of pigments seems probable when observations are made as regards their behaviour towards various reagents. The sub-classes will be described later.

(2) Soluble yellow pigments known as "*xantheïn*." Again, various sub-classes, to be described later, may be made according to their reactions towards reagents.

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* General classifications and properties of pigments, as far as they are known, are given by Czapek (4) and Zimmermann (13).

† Said to be occasionally precipitated or crystallised out on natural concentration of the cell-sap.

B. Pigments associated with specialised protoplasmic bodies—chromoplastids—the colour in this case being usually yellow, orange-yellow, orange, or orange-red. Insolubility in water appears to be a constant characteristic of this group. Two well-known pigments are included here :—

(1) *Carotin*—a hydrocarbon of definite and characteristic properties. According to Zimmermann (13), it is insoluble in water, almost so in alcohol, slightly soluble in ether, more readily in benzine, and most so in chloroform and carbon bisulphide. It occurs naturally and can be obtained artificially in crystalline form, and is microchemically recognised by certain reagents; with concentrated sulphuric acid it gives an indigo-blue colour, at first momentarily violet, and with iodine a green or greenish-blue colour.

(2) *Xanthin*—again, according to Zimmermann (13), occurs in the plastid in amorphous form. It is insoluble in water, somewhat soluble in ether, chloroform, and benzine, but more so in alcohol. Microchemically xanthin can be recognised by giving with concentrated sulphuric acid a blue colour, at first momentarily green, and with iodine a green colour.

In addition to the above, there appear to be other plastic pigments, which do not give a blue colour with sulphuric acid, but a yellow or brown.

Anthocyanin.

Classification of the soluble red-purple-blue pigments has always been a difficult problem, but there seems to be evidence that anthocyanin is a general term, including several different pigments. The differentiation made here is based on the inheritance of colour in certain genera, on the sequence of flower-colour in cultivated varieties, and finally on the behaviour of red pigments towards chemical reagents.

There are present in most plants colourless or pale yellow substances, soluble in water, but insoluble in ether; with strong acids and alkalis they give a canary-yellow colour, and a similar coloration or most frequently a precipitate of the same colour with basic lead acetate. In some cases, *Eschscholtzia californica* (Courchet (3)), *Argemone grandiflora*, and yellow species of *Viola*, these substances crystallise from extract solutions in needle-shaped crystals aggregated in clusters or spherules; solutions of the crystals from the above genera reduced Fehling's solution slightly, but after prolonged boiling with dilute acid, a deep yellow substance, together with a reduction of Fehling's solution, was obtained, suggesting the glucoside nature of the crystalline bodies. A similar glucoside is probably present in *Narcissus Tazetta* (Bidgood (2)).

The colour reaction with alkalis and acids is most obvious in parts free from chlorophyll; in unpigmented genera, *Galanthus nivalis*, for instance, or in pigmented (anthocyanic) genera such as many *Umbelliferæ*, with white flowers, the petals turn bright canary yellow with ammonia.

The inheritance of colour in *Antirrhinum* (Wheldale (12)) has led to the suggestion that anthocyanin is possibly a compound of such a glucoside-like body with a reddening substance. The original type of *Antirrhinum* has magenta (anthocyanin) flowers.* Loss of the reddening substance, which may be represented by a Mendelian factor (M), gives a variety bearing ivory-white flowers containing no pigment (except in the palate and hairs), but a glucoside-like body giving the reactions with acids, alkalis, and lead acetate described above. Further loss of a substance, again represented by a factor (I), from the glucoside-like body in the superficial cells of the lips gives a yellow xantheïc pigment, and the variety thus bears yellow flowers. Loss of yellow pigment, represented by yet another factor (Y), gives an albino, containing no pigment and no glucoside-like body. Local decomposition produces the same xantheïc pigment on the palate and in hairs on the inner surface of the tube in all varieties except the albino. The albino may carry I or M, or both, since these factors are invisible unless the fundamental colour Y is present. Moreover, the reddening factor can exist with Y, the decomposition product, giving a mixed colour, *i.e.*, crimson. Each variety may breed true or may throw itself and one or more varieties below it in the scale of colour, according as it is homo- or heterozygous in the various factors. Magenta can throw all varieties; crimson can throw yellow and white; ivory, yellow and white; and yellow, white only.

At this point it is interesting, perhaps, to give views regarding the constitution of anthocyanin, based on results obtained from a totally different kind of investigation. Overton (9) found that in plants supplied artificially with excess of sugar and other carbohydrates there is a correlated increase in the production of anthocyanin, and he concludes that the latter, in many cases, is a glucoside compound of a tannic acid. Molisch (8) and Heise (6) are also of the opinion that some red sap-pigments are glucosidal in nature. Tannin is by no means always present in plants containing anthocyanin; the magenta pigment of *Antirrhinum* gives a tannin reaction, but no tannin has been found in the albinos.

A similar range of colour to that found in *Antirrhinum*, *i.e.*, various shades of purple or magenta and crimson, together with ivory, yellow, and sometimes white, also occurs in *Althæa rosea*, *Azalea*, *Dahlia variabilis*, *Dianthus Caryophyllus*, *Helichrysum bracteatum*, *Linaria*, *Nemesia*, *Phlox Drummondii*, and *Rosa.* It is probable that the inheritance of flower-colour will be found to be similar in these genera.

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* With yellow pigment locally on the palate and in hairs on the inner surface of the tube.

The inheritance of colour in *Lathyrus* and *Matthiola* (Bateson, Punnett and Saunders (1)) differs from *Antirrhinum,* in that two factors (C and R) are required to produce colour, and the loss of either gives a white containing only a glucoside-like substance. An additional factor, B, gives the purple varieties.

A similar range of colour to that in *Lathyrus, i.e.*, shades of blue or purple and red, together with white, is shown by *Campanula, Digitalis purpurea, Iberis, Lobelia, Nemophila insignis, Pisum,* and many others; no xantheïc variety occurs in this series.

It is evident, then, that there are two classes of anthocyanin as regards the series of varieties to which each can give rise; in one case the decomposition possibly of the glucoside-like constituent gives a yellow xantheïc form, and in the other case no such decomposition is possible, and no yellow variety exists.

The close relationship between xantheïc pigments, the glucoside-like bodies, from which they may be derived, and the anthocyanin, of which these bodies are themselves possibly constituents, is suggested also by the fact that yellow xantheïc varieties almost always have an anthocyanic type. This connection is well exemplified among the genera of the Compositæ. Usually plastid pigments in addition are present in this order, but these may be disregarded for the moment. Yellow varieties of *Coreopsis, Chrysanthemum carinatum, Dahlia variabilis, Helichrysum bracteatum,* contain xantheïn, while the type has anthocyanin; other genera, *Zinnia elegans, Gaillardia, Hieracium rubrum,* have anthocyanin of the kind which gives no xantheïc varieties, while, finally, *Calendula officinalis, Helianthus annuus, Erigeron* spp., and *Senecio* spp. have no xantheïn and no anthocyanin.

When we consider the behaviour of anthocyanin towards such substances as acids, alkalis, certain salts, etc., we find this term includes at least several groups of pigments. Two classes have been described by Weigert (11), and termed "weinrot" and "rübenrot" respectively. The former is soluble in both alcohol and water, giving with basic lead acetate blue-grey or blue-green precipitates, and with concentrated sulphuric acid a bright red colour. It is found in the leaves of *Vitis, Ampelopsis quinquefolia, Rhus typhina, Cornus sanguinea,* and others. The latter, rübenrot, Weigert found in leaves of the *Amarantaceæ* and *Chenopodiaceæ* (*Beta vulgaris, Iresine, Amaranthus, Atriplex*), and in fruits of *Phytolacca decandra.* Though readily soluble in water, the pigment is insoluble in alcohol; with basic lead acetate it gives a red precipitate, with sulphuric acid and with ammonia a deep violet, but with other bases a yellow colour. He further distinguishes a pigment, "Malvenviolett," occurring in the leaves of *Coleus Hero, Perilla nankinensis, Corylus avellana atropurp.*, red *Brassica,* and *Malva* sp. It is present as a compound, which, on the action of acids, produces the weinrot pigment.

In the present paper, conclusions are based upon the examination of relatively few red pigments, and even these present great complexity. Yet, on the whole, a rough classification may be given as follows :—

1. A purple anthocyanin, which is characterised by giving a deep blue or violet colour with ferrous sulphate and ferric chloride, a green colour with alkalis and a blue or blue-green precipitate with basic lead acetate. Such a pigment is found in the deep purple and crimson varieties of *Dianthus Caryophyllus, Lathyrus odoratus, Phlox Drummondii,* the purple and plum varieties of *Matthiola* (Saunders (1)), purple *Fuchsia,* and in the berries of *Atropa Belladonna* and *Rosa pimpinellifolia.*

2. A purplish-red anthocyanin, corresponding probably to Weigert's weinrot. This pigment does not give the above reaction with iron salts; but with alkalis a green colour and a green precipitate with basic lead acetate. This form occurs in the magenta varieties of *Antirrhinum majus, Dahlia variabilis,* pale magenta and violet varieties of *Phlox Drummondii,* red varieties of *Lathyrus odoratus, Salvia Horminum,* and *Verbena*; also in the crimson *Cheiranthus Cheiri,* in *Tradescantia virginiana* and in *Rhus aromatica.*

3. A red anthocyanin characterised by giving a red precipitate with basic lead acetate. Two sub-groups may be further identified as—(*a*) those giving green colour with alkalis—red, copper, and rose varieties of *Matthiola,* and a red variety of *Delphinium*; (*b*) those giving a reddish yellow colour with alkalis—pink varieties of *Dianthus Caryophyllus,* flesh and terra-cotta varieties of *Matthiola,* certain scarlets of *Lathyrus,* salmon-rose varieties of *Dianthus barbatus, Phlox Drummondii, Verbena,* and the "rose dorée" variety of *Antirrhinum majus.*

The above classes seem to show a gradual diminution in the amount of blueness present as we pass from the very blue purples of *Lathyrus,* through the magentas and blue-reds of *Antirrhinum* and *Lathyrus,* to end finally in such varieties as the "rose dorée" *Antirrhinum,* from which blueness is practically absent. The bluer form is usually the original type and the reds, as derivatives, are probably components of the original anthocyanin.

There is some indication of a connection between the chemical behaviour of the classes and their inheritance. The purple anthocyanin appears to be that form, which, in *Lathyrus* and *Matthiola,* is given when the B factor is present in addition to C and R. The extreme purple is not found in *Antirrhinum*; hence in the latter case, when a blueing factor is present, as in the magenta type, purplish-red anthocyanin is the ultimate form. The red of *Antirrhinum* is represented by the "rose dorée" type, which finds its parallel in the flesh and copper of *Matthiola*, certain scarlets of *Lathyrus*, and the salmon-rose of *Phlox Drummondii*.

It is of interest to note also that in the reds of *Antirrhinum* and the salmon-rose of *Phlox*,* of which several shades exist, the deeper are dominant to the paler, while in the purples, purple-reds, and magentas of *Lathyrus Matthiola*, *Phlox*, and *Antirrhinum*, the paler shades are dominant to the deeper. As regards shades of one colour, a fuller investigation has been made in *Antirrhinum*, in which genus every shade is a definite zygotic form, and the chemical reactions of these shades are fundamentally similar though differing in degree. The precipitates with basic lead acetate, for instance, are of varying shades of green, yet these remain unaltered on artificial concentration or dilution of the extract. This would appear to indicate that the shade of colour was but an outward indication of some definite organic compound in the sap.

All the red pigments so far described give, with strong sulphuric acid,† bright red and yellow colours, becoming orange when mixed; it seems possible that the red is due to the reddening factor, and the yellow to the glucoside-like constituent of the anthocyanin. With alkalis, the reddening factor of the bluish-red turns blue and the other body yellow, the result being green, sometimes rapidly fading to yellow. Basic lead acetate gives blue-green or green precipitates, due again to the same mixture.

The scarlet pigment of some genera—*Lobelia cardinalis*, *Phaseolus multiflorus*—is again different, in that it gives a bluish colour with alkalis and a red precipitate with basic lead acetate.

With regard to natural orders and relationships, as far as these investigations have gone, it appears that the red pigments of the *Papaveraceæ* differ from others. Also those of the allied orders *Amarantaceæ*, *Nyctaginaceæ*, *Phytolaccaceæ*, and *Portulacaceæ* (included in the *Centrospermæ* by Engler), form an isolated group giving reactions essentially different from any hitherto described.

Of the *Papaveraceæ*, the red pigment of *Glaucium phœniceum* and *Papaver Rhœas* gives a purple colour with basic lead acetate.

The red pigment of *Amaranthus* (and other genera of the same order according to Weigert (11)) is characterised by its insolubility in alcohol. With concentrated sulphuric acid it gives a purple colour, with ammonia a reddish colour, but with other bases a clear yellow, and with basic lead acetate an orange-red precipitate. In the stems and berries of *Phytolacca decandra*, the magenta and crimson flowers of *Mirabilis Jalapa* and *Portulaca grandiflora*, two pigments apparently exist. One, insoluble in alcohol but soluble in water to a magenta solution, gives reactions on the whole similar to those given by the red pigment of *Amaranthus*. The other pigment is soluble in alcohol to a crimson solution, which gives a yellow colour with acids and alkalis and a reddish precipitate with basic lead acetate.

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* I am indebted to Miss Killby for this information.

† Reactions are best seen by dropping acid on to the pigment on a white porcelain plate. Mixtures of colours are thus more readily detected than in bulk in solution.

Overton (9) is also of the opinion that anthocyanin contains several classes of pigments, of which he gives—(1) the *Amarantaceæ* and *Beta vulgaris*, (2) *Papaver Rhœas* and other species of *Papaveraceæ*, (3) *Tradescantia discolor** and other *Commelinaceæ*.

The alcoholic solution of the red pigment in some genera is colourless, the colour returning on evaporation or on addition of acid. In other cases, again, the alcoholic solution is as deeply coloured as the flowers. It is possible that these phenomena may indicate a difference in the nature of the pigments.

Lastly, it might be well to mention that anthocyanin is said to occur in solid and crystalline states in the cell. Many instances are cited by Mölisch (8) in his work on crystalline anthocyanin.

Xantheïn.

Xantheïn, like anthocyanin, includes at least several pigments varying in their reactions towards acids and alkalis. They may be classified as follows:—

1. Those giving a deeper yellow, orange, or orange-red colour with acids and alkalis and similarly coloured precipitates with basic lead acetate. Such pigments are found in yellow varieties of *Althœa rosea*, *Antirrhinum majus*, *Calceolaria*, *Coreopsis*, *Dahlia variabilis*, *Dianthus Caryophyllus*, *Helichrysum bracteatum*, *Phlox Drummondii* and *Tagetes signata*.

2. Those in which the yellow colour becomes paler with acids and alkalis and basic lead acetate gives, as a rule, no precipitate or a precipitate of the same colour as the pigment. Such is the case in *Mirabilis Jalapa*, *Montbretia* sp., *Nemesia strumosa*, *Papaver nudicaule*, and *Portulaca grandiflora*.

3. Those in which the yellow colour remains unaltered in the presence of acids and alkalis, and basic lead acetate gives a yellow precipitate, as in *Mesembryanthemum pomeridianum*, *Verbascum*.

If xantheïc pigments are derivatives of anthocyanin, the dissimilarity of the former among themselves strengthens the view that the reds, from which they are derived, are also dissimilar.

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* I have not been able to detect so far any divergence in this case.

Albinism.

It has been suggested that there are two forms of anthocyanin giving respectively two colour series, one containing a yellow xantheïc variety, the other not. Whites occur in both, and it seems probable that the term albinism should be used in a different sense when applied to each of the two series.

The extract from most white flowers (also from flowers coloured only with plastid pigments, when these have been removed), gives a canary colour with strong acids and alkalis, as stated previously. Without exception, as far as observations have gone, whites of genera having no yellow sap type, have given this yellow colour-reaction. These whites may, without hesitation, be declared to be recessive to the red-purple-blue types, and they are albinos as regards anthocyanin.

On the other hand, in the case of *Antirrhinum*, *Azalea* and *Phlox Drummondii*,* belonging to the series giving yellow sap-colour, whites exist which do not give the same yellow colour-reaction. Moreover, these whites are recessive to yellow in *Antirrhinum* and *Phlox*, and are albinos as regards both anthocyanin and xantheïn. It is the ivory in this series which contains the glucoside-like body, and gives the yellow colour-reaction.

Whites giving no colour-reaction have not yet been observed in the other genera mentioned in the anthocyanic-xantheïc series, though relatively few types have been examined. It is possible that the true albino, as contrasted with ivory, is rare in commercial samples, since the albino type in *Antirrhinum* and *Phlox* has been found to set poor seed unless fertilised artificially.

What appears at first an exception to this view is the case of *Mirabilis Jalapa*. Here we find a range of colour similar to that in *Antirrhinum*, *i.e.*, shades of magenta and crimson, together with deep and pale yellow and white. The white (when it does not carry a reddening factor) is recessive to yellow,† and yet gives a colour-reaction with ammonia, etc. The explanation lies in the fact that both the yellow and red pigments in *Mirabilis* (see p. 50) are of an entirely different nature from those in *Antirrhinum* and *Phlox*. For the same reason, the inheritance in *Portulaca grandiflora* will, if worked out, doubtless prove to be similar to *Mirabilis*.

Shull (10) also gives the case of *Verbascum Blattaria*, in which a very pale form, perhaps an albino, is recessive to the full yellow. He also states that Correns found white *Polemonium cœruleum* to be dominant to yellow *Polemonium flavum*. If the former contained a glucoside-like body, and the latter a xantheïc pigment, the result might be analogous to *Phlox*. As material of these genera was not available, the pigments have not been investigated, and consequently this conjecture must remain unverified for the present.

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* I am indebted to Miss Killby for the information that in *Phlox Drummondii* the ivory type may throw both the yellow and the albino.

† I am indebted to Miss Marryat for this information from results obtained in cross-breeding of *Mirabilis*.

Plastid Pigments.

The plastid pigments, carotin and xanthin, are well-known substances, of which the properties and characteristics have been investigated. Both may be present in the same plastid, when the colour is orange-yellow, orange or orange-red, and this condition is very widely distributed; or xanthin only may be present, when the colour is yellow. In the orange-yellow or red type the loss of the power to produce carotin in the plant may give rise to a lemon-yellow variety. This is the case in flowers of *Argemone grandiflora*, *Calendula officinalis*, *Tagetes signata*, *Tropæolum majus*, and probably *Cheiranthus Cheiri* and *Salpiglossis grandiflora*.

In other cultivated genera, where the type contains xanthin, this pigment appears to give rise to paler yellow varieties containing derivative plastid pigments, probably decomposition products of xanthin, and giving a yellow or brown colour with strong sulphuric acid. At present these derivatives have not been thoroughly examined. They are found in the pale yellow varieties of *Helianthemum* spp., *Chrysanthemum carinatum*, in the autumn cultivated *Chrysanthemum*, and in *Zinnia elegans*.

There is evidence from cross-breeding in *Cheiranthus* and *Tropæolum** that presence of carotin is dominant to its absence, that is the orange-yellow variety is dominant to the lemon-yellow.

The plastid pigment in cream varieties of *Lathyrus odoratus*, *Matthiola*, *Rosa*, and *Eschscholtzia caniculata rosea*, is again different from carotin and xanthin as regards its chemical reactions. There is evidence from cross-breeding in *Lathyrus* and *Matthiola* (Bateson and Saunders (1)) that cream plastid pigment is recessive to its absence, *i.e.*, colourless plastid.

Combinations of Soluble and Plastid Pigments.

Anthocyanin and plastid pigments are frequently found together in plants. When the red sap occurs with plastids containing both carotin and xanthin, the resulting colour is some shade of brown, crimson, or orange-red; with plastids containing xanthin only, or some derivative product of xanthin the resulting colour is maroon, purple, or salmon-pink. Hence we find in cultivated genera containing plastid pigments and anthocyanin a colour series brown, crimson, or orange, purple, magenta, or salmon-pink, deep yellow and pale yellow.

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* I am indebted to Miss Saunders for information regarding *Tropæolum*.

Such is the case in *Cheiranthus Cheiri*, *Chrysanthemum* spp., *Helianthemum* spp., *Salpiglossis grandiflora*, *Tagetes signata*, *Tropæolum majus*, and *Zinnia* spp.

This series differs from the anthocyanic-xantheïc series in one respect; in the former the type is crimson and the purple or magenta is the derivative, whereas in the latter the purple or magenta is the type, while crimson is a derivative. These two contrasting series cannot be better exemplified than by the two indigenous genera, *Antirrhinum* and *Cheiranthus*, and their cultivated varieties. The wild *Cheiranthus* is deep yellow tinged with brown; cultivation has produced from the original, a pale yellow type, to which the addition of anthocyanin gives purple. The wild *Antirrhinum* is magenta, which, on loss of some constituent, has given a yellow xantheïc type, and this gives, further, in presence of the reddening substance, a crimson.

Stress should be laid, in connection with colour, on the conception of the pigmentation of a plant as a whole. The power to produce colour is the property of every cell of a pigmented plant; frequently the flowers are white or show but little colour in plants which are really pigmented, as, for example, *Solanum nigrum*, *Geranium Robertianum*, var. *album*. In a plant having red, purple, or blue flowers, anthocyanin may invariably be detected in the vegetative parts, such as cotyledons, under surfaces of leaves, wounded or exposed areas, etc. The diffusion of colour throughout the plant is manifested in the correlation so frequently found between fruit- and seed-colour on the one hand and flower-colour on the other. De Vries (5) gives as examples the green-flowered variety of *Atropa Belladonna* and the white-flowered variety of *Daphne Mezereum* with yellow fruits; also the white-flowered *Linum* with yellow seeds as contrasted with the brown seeds of the blue variety. The colour and pattern of seed-coat in *Matthiola* (Bateson and Saunders (1)) and Pisum (Lock (7)) is also correlated with flower-colour in the same way.

Method for Examination of Pigments.

The material to be examined is ground very finely with powdered glass in a mortar, extracted with methylated spirit and filtered. If from the colour of the residue, or from a microscopic examination, the presence of carotin be suspected, a further extraction is made with benzine or chloroform. The alcohol extract contains the pigments soluble in water and such plastid pigments as are soluble in alcohol (chiefly xanthin). After evaporation to dryness on a water-bath, the xanthin is separated by ether from the xantheïc and anthocyanic pigments. The chloroform extract contains both xanthin and carotin; the latter can be washed free from xanthin by means of alcohol.

DETAILS FOR GENERA OF VARIOUS NATURAL ORDERS.

The following particulars chiefly concern yellows, the reds having been dealt with in detail previously.

Aizoaceæ.

Mesembryanthemum pomeridianum has a xantheïc pigment, unaffected by acids and alkalis and precipitated by basic lead acetate.

Amaryllidaceæ.

Alstrœmeria aurantiaca has plastids containing carotin and xanthin; anthocyanin is present in addition.

Caryophyllaceæ.

Dianthus Caryophyllus, the parent form of the Carnation, shows the anthocyanic-xantheïc series. The yellow pigment is intensified to orange yellow by acids and alkalis. Magentas and crimsons are probably produced by the addition of red sap to ivory and yellow respectively. Ivory gives the yellow colour-reaction with acids and alkalis, but whether it is dominant to yellow, and whether an albino, recessive to yellow, exists has not yet been ascertained.

Cistaceæ.

Varieties of *Helianthemum vulgare* show the anthocyanin-xanthin series and pale yellows, which are derivative plastid pigments, probably from xanthin. The type is crimson, *i.e.* anthocyanin on xanthin, and the magenta and pink types are due to the same sap-colour on the pale yellow derivative forms.

Compositæ.

Calendula officinalis exists in two varieties, orange and lemon yellow; in the former the colour is due to plastids containing carotin and xanthin, in the latter xanthin only. There is apparently no anthocyanic form.

Chrysanthemum carinatum shows the anthocyanic-xantheïc-xanthin series with pale yellows containing derivative products of xanthin, and whites giving the yellow colour-reaction with acids and alkalis, and containing colourless plastids. Anthocyanin on whites, pale yellows, and deep yellows gives the usual magentas and crimsons. In the yellows, from which anthocyanin is absent, a xantheïc pigment exists, which gives an orange colour with acids and alkalis and an orange precipitate with basic lead acetate.

The autumnal cultivated forms of *Chrysanthemum* resemble the above species, except that the anthocyanin appears to be of the kind which does not give a xantheïc derivative.

Coreopsis Drummondii has a brown patch of anthocyanin-containing cells at the base of the ray-florets. The orange-yellow of the florets is due to plastids containing carotin and xanthin. A xantheïc pigment, probably left as the anthocyanin retreats to the base of the florets, is also present in their upper portions; it turns orange-red with acids and alkalis and is precipitated by basic lead acetate as an orange precipitate.

Dahlia variabilis shows the anthocyanic-xantheïc series. The yellow turns a brilliant orange colour with acids and alkalis, and is precipitated as a deep orange-red precipitate by basic lead acetate. The ivory gives the yellow colour-reaction. Magentas and purples are anthocyanin on ivory, and crimsons are anthocyanin with xantheïn.

Gaillardia spp. have usually orange-yellow ray-florets, with anthocyanin at the base. The orange-yellow is due to plastids containing carotin and xanthin. There appears to be no xantheïc derivative.

Gazania splendens has orange-yellow ray-florets with a dark basal patch of anthocyanin cells. Orange-yellow is again due to carotin and xanthin.

Helianthus annuus has plastids containing xanthin; a pale yellow variety exists in which the plastids probably contain some derivative product of xanthin.

Helichrysum bracteatum shows the anthocyanic-xantheïc series. The white variety gives the yellow colour-reaction with acids and alkalis. The yellow contains a xantheïc pigment, which gives an intense orange colour with acids and alkalis, and a similarly coloured precipitate with basic lead acetate. Magentas and crimsons are due to anthocyanin on white and yellow respectively.

Hieracium rubrum has anthocyanin in addition to plastids containing carotin and xanthin.

Picris pauciflorus, Senecio Jacobæa, and *Taraxacum officinale* have only plastids containing carotin and xanthin.

Tagetes signata shows the anthocyanin-xantheïn-plastid series. There is an orange-yellow variety with plastids containing carotin and xanthin, and a lemon-yellow variety with plastids containing xanthin only; both yellows have in addition a xantheïc pigment derived from anthocyanin; it is intensified in colour by acids and alkalis, and gives an orange precipitate with basic lead acetate. Anthocyanin on the deep yellow gives brown; on the lemon-yellow maroon.

Zinnia elegans shows the anthocyanin-xanthin series, with pale yellow derivative plastids from xanthin. Anthocyanin gives the usual magentas, pinks, crimsons, and orange on the pale and deep yellows.

Cruciferæ.

Brassica sinapis has plastids containing xanthin.

Cheiranthus Cheiri has two yellow varieties, a deep and a pale; the former has plastids containing both carotin and xanthin. It is probable that the pale yellow contains xanthin only, though it has not been tested. Anthocyanin gives the crimson or brown on the deep yellow, and the purple varieties on pale yellow respectively.

Matthiola shows the anthocyanin series with purples, reds, and whites, the latter having colourless plastids; anthocyanin may also exist with plastids containing a "cream" pigment differing from both carotin and xanthin.

Cucurbitaceæ.

Cucurbita Pepo has plastids containing carotin and xanthin.

Fumariaceæ.

Corydalis lutea appears to have plastids containing xanthin and a yellow xantheïc pigment precipitated by basic lead acetate as an orange-yellow precipitate.

Hypericaceæ.

Hypericum Hookerianum has plastids containing xanthin.

Leguminosæ.

Coronilla viminalis has plastids containing carotin and xanthin; some anthocyanin is also present.

Lathyrus odoratus shows the anthocyanin range, purple, red, and white. Also a "cream" plastid pigment similar to that in *Matthiola.*

Spartium junceum has plastids containing carotin (?) and xanthin.

Liliaceæ.

A variety of *Lilium tigrinum* was found to contain plastids and anthocyanin. From the plastids a brick-red pigment was extracted giving a blue colour with sulphuric acid, though no purple was detected; it is probably a form of carotin.

Linaceæ.

Linum flavum has plastids containing carotin and xanthin.

Loasaceæ.

Bartonia aurea has plastids containing carotin and xanthin.

Malvaceæ.

Althæa rosea shows the anthocyanic-xantheïc series. The yellow pigment is intensified in colour by acids and alkalis and is precipitated by basic lead acetate as a brownish-orange precipitate. The ivory gives the yellow colour-reaction. It has not yet been ascertained whether the yellow is recessive to ivory nor whether an albino exists. Anthocyanin gives various purples, mauves, magentas, pinks, crimsons, and orange, according as it is present on an ivory or on a yellow ground.

Nyctaginaceæ.

Mirabilis Jalapa shows the anthocyanic-xantheïc series. The yellow pigment becomes paler with acids and alkalis, and is not precipitated by lead acetate. The white variety gives the yellow colour-reaction, but is recessive to yellow. The red pigment, as already stated, differs from most other forms of anthocyanin.

Onagraceæ.

Œnothera Lamarckiana has plastids containing xanthin and, in addition, a pale yellow xantheïc pigment intensified to orange-yellow by acids and alkalis.

Papaveraceæ.

Argemone grandiflora exists in three varieties, deep and pale yellow and white. The deep yellow is due to plastids containing carotin and xanthin; in addition, a crystalline glucoside, similar to that in *Eschscholtzia,* is present in the sap. The pale yellow appears to contain xanthin and the glucoside, and the white gives the yellow colour-reaction.

Eschscholtzia californica has plastids containing both carotin and xanthin; sometimes the margin or outer half of the petals is yellow or the orange petal is striped with yellow. Examined microscopically the yellow colour of the streaks and margin is seen to be due to yellow plastids, containing, undoubtedly, xanthin only. The orange portions contain orange plastids, carotin being present in addition in these. The sap contains a glucoside (the soluble yellow pigment of Courchet (3)), crystallising in spherules of needles. *E. Caniculata rosea* is cream tinged with pink. Plastids are present containing only a little xanthin; most of the plastid pigment appears to be similar to that in cream *Matthiola.* The glucoside is also present, though in smaller quantity than in the orange species. The pink tinge is due to anthocyanin.

Glaucium luteum has plastids containing carotin and xanthin.

G. phœniceum, an orange-red species, has anthocyanin in addition.

Papaver nudicaule exists in three varieties, orange, yellow and ivory-white. The pigment appears to be of the xantheïc type, which becomes paler on addition of acids and alkalis, and is not precipitated by lead acetate.

Polemoniaceæ.

Phlox Drummondii has already been considered. The xantheïc pigment is deepened by acids and alkalis and precipitated by basic lead acetate as a deep yellow precipitate. The ivory gives the yellow colour-reaction, and the albino, which does not give this reaction, is recessive to yellow.

Ranunculaceæ.

Ranunculus spp. have plastids containing carotin and xanthin.

Rosaceæ.

Potentilla fruticosa has plastids containing xanthin.

Cream *Rosa* spp. contain a plastid pigment similar to that in cream *Matthiola.*

Scrophulariaceæ.

Antirrhinum has already been fully described.

Calceolaria spp. contain a xantheïc pigment intensified in colour by acids and alkalis, and precipitated by basic lead acetate as an orange-brown precipitate. In some cases plastids containing xanthin are also present.

Varieties of yellow *Nemesia strumosa* have a xantheïc pigment, which becomes paler on treatment by acids and alkalis.

Verbascum Lychnites has a xantheïc pigment giving a blue colour with sulphuric acid, fading to yellow, a yellow colour with alkalis, and a yellow precipitate with basic lead acetate.

Solanaceæ.

Hyoscyamus Chloranthus, a yellow species devoid of purple veining, has plastids containing xanthin.

Physalis Alkekengi has plastids containing carotin and xanthin in the orange calyx.

Salpiglossis grandiflora shows the anthocyanin-plastid series. The deep yellow is due to plastids containing carotin and xanthin; the pale yellow probably contains xanthin only. Various purples and crimsons are given on the addition of anthocyanin.

Tropæolaceæ.

Tropæolum majus shows the anthocyanin-plastid series. In the pale yellow variety the plastids contain only xanthin, in the deep yellows carotin and xanthin. Anthocyanin may be present in addition at the base of the petals or diffused throughout the flower. Various concentrations of anthocyanin on pale and deep yellow give orange-red, salmon-red, crimson, etc.

T. canariense has plastids containing xanthin only.

T. speciosum contains anthocyanin.

Violaceæ.

Viola tricolor shows the anthocyanin-plastid series. The deep yellow has plastids containing carotin and xanthin, and in addition a crystalline glucoside, similar to that found in *Eschscholtzia.* A paler yellow variety appeared to contain the glucoside only.

Summary of Results.

1. "Anthocyanin," the term used in connection with the red sap-colour in plants, includes several pigments differing as regards their inheritance, the colours to which they give rise in variation, and their behaviour towards chemical reagents.

2. The colours of the varieties arising from an "anthocyanic" type may be regarded as components of the original "anthocyanin"; the type, conversely, may be supposed to lose its components (which are expressible as Mendelian factors) in succession, thus giving rise to a series of colour variations.

3. Broadly speaking, there are two series of colour variations, one containing a "xantheïc" derivative, as, for example, in *Antirrhinum majus*, the other no such derivative, as in *Lathyrus odoratus.*

4. Albinism, in the first series, is a lack of both "anthocyanin" and "xantheïn"; in the second series of "anthocyanin" only.

5. "Xantheïn," a term used in connection with yellow sap-colour, includes several different pigments. This is to be expected if the view that "xantheïn" is a derivative of "anthocyanin" be accepted.

6. There is evidence, as far as investigations have gone, of a correlation between the behaviour of pigments in genetics and their reactions towards chemical reagents.

7. In the case of plastid pigments, the type may contain carotin, xanthin, or both. Varieties arise in some cases from loss of power to

produce carotin, or in others from loss probably of some of the constituents of xanthin.

8. "Anthocyanin" may exist together with plastid pigments in the type, in which case derivative products of both forms of pigmentation are found among the varieties.

I wish to take this opportunity to express my sincere thanks to Prof. Bateson for his kindness and help throughout this research. I am also indebted to Mr. John Parkin, who has kindly made various suggestions.

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An Experimental Estimation of the Theory of Ancestral Contributions in Heredity.

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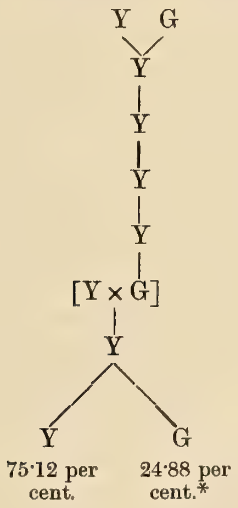
(Communicated by J. Bretland Farmer, F.R.S. Received December 7, 1908,—Read February 11, 1909.)

The experiments described in the following pages were undertaken with the object of finding out if the proportions in which characters segregate in the F_2 generation are affected by the distribution of those characters over the parentage and ancestry of the forms crossed. To this end I crossed a yellow pea with an extracted green in F_5, and obtained a purely negative result. The proportion of yellows and greens in the F_2 generation from this cross did not differ sensibly from that obtaining in the F_2 generation from a cross between a pure yellow and a pure green. The nature and result of the experiment may be summarised in the following pedigree, in which the two forms which I crossed are enclosed in brackets :—



Details of the Experiment.

The Parent Forms.—(i) *The Greens.*

The original cross which provided me with the extracted F_5 greens used in my experiment was made by Mr. C. C. Hurst, and has been fully described by him.* In the spring of 1905, Mr. Hurst kindly sent me some seeds, the cotyledons of which belonged to the F_3 generation resulting from the cross which he had made. All the seeds were borne on a single plant (No. 31), of which the following is the pedigree, which is copied exactly from the MS. pedigree which Mr. Hurst sent to me with the seeds:—

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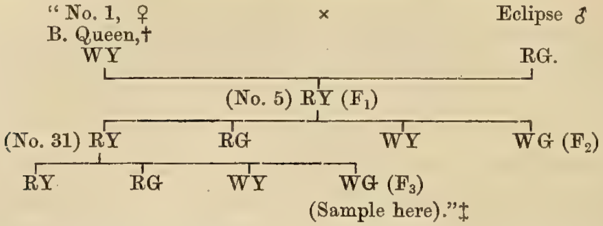
* See Summary on p. 78.



I sowed the yellow round seeds of the sample which Mr. Hurst sent me (*i.e.*, the YR cotyledoned plants of the F_3 generation) in the spring of 1905, and harvested F_4 in the autumn of that year. Of this F_4 generation only the YR were, as before, sown in the spring of 1906, and F_5§ was harvested in the autumn of that year. I kept some green round seeds from three plants of this generation, and it was this seed which produced the plants on which I made the crosses the results of which are recorded in this paper. The characters of these three plants are summarised in the following table, which gives the catalogue numbers of the parent plants and of the "green" plants‖ raised from their seeds, the numbers of the various kinds of seeds borne on the three parent plants, etc.:—

Catalogue numbers of—		Number of seeds borne by parent plant.				Number of green seeds sown.	Number of plants raised.
Parent plant.	Sowing of green seeds borne on it.	YR.	YW.	GR.	GW.		
B55.13.........	483	58	—	21	—	7	7
B54.180	484	29	9	7	2	6	5
B54.183	485	41	—	14	—	12	11

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* 'Journ. R. Horticultural Soc.,' vol. 28, pp. 483—494.

† British Queen.

‡ Y signifies yellow, G green, R round, and W wrinkled. Mr. Hurst writes the shape of the seed first. Throughout the present paper, however, the colour is written first.

§ The proportions of "yellows," "greens," "rounds," and "wrinkleds" in these generations will be published at a later date.

‖ By a "green" plant I mean one raised from a seed with green cotyledons.

(ii) *The Yellows.*

I shall now give a brief account of the various strains of yellow peas used in the experiment. I had been endeavouring for some time previous to 1907 to bring together a collection of peas from widely separated sources, with a view to repeating the observations of Mendel and others on a broader basis of material. The yellow strains thus obtained were used in this experiment. I shall briefly describe them in the order in which they occur in my records, writing the catalogue number before the description of them.

(477) This is a native Chinese cultivated pea, and was procured for me in a village not far from Pekin by Dr. Korsakov, of the Russian Legation in Pekin. *Wen-dou* is the name written on the packet in which they were sent. The peas are yellow and round. The pod and the peas themselves are very small, and have evidently not been imported from Europe during the last half century, if, indeed, there is any reason to suppose that they have been imported at all.

(478) This is one of a set of peas which my colleague, Mr. F. J. Bridgman, procured from a shipping merchant who was kind enough to put up a few packets of seeds from cargoes which had been shipped from various parts of the world. 478 was a particularly fine seed, resembling Victoria marrow, from a cargo which had been shipped from Germany.

(479) Nineteen seeds of this variety came into my possession at a meeting of the Natural History Society at the Royal College of Science during December, 1906. It is a small yellow round pea.

(480) From Calcutta.
(481) From Canada.
(482) From Russia.
} Through the same channel as 478. All yellow round.

(492) A yellow round pea which I bought in Genoa in April, 1906. The parents of the plants which were used in this experiment were grown in my garden in 1907. A yellow round pea.

(493) The same as 492, from a later sowing in 1907.

Explanation of Tables I—VIII.

Cross-pollination was effected in the following way:—A flower of suitable age was selected to function as pistil-parent; one side of its carina was torn transversely, the whole carina then temporarily slipped off the bunch of stamens, which were removed with forceps, after it had been determined that they had not yet dehisced. A flower of suitable age to function as pollen-parent was selected and plucked from the plant which bore it; the tip of its carina was cut off and the stigma of the pistil-parent was inserted into the aperture in the carina made by the cut, and consequently into the mass of pollen which it contained. The carina of the pistil-parent was then slipped back, and the standard and wings folded into their original position. The flower was then labelled. As a rule, I pollinated four flowers with the pollen of a single flower, in order to save time. When more than one flower has been pollinated from a single flower, this fact is indicated in Tables I and II by writing all the crosses made with the pollen of a single flower on the same line ; except in cases in which different kinds of pistil-parents were fertilised from the same flower, when they are connected by a vertical bracket, as in crosses 121—124 (Table I).

The flowers of the pistil-parent were not enclosed in bags after they had been pollinated. I did not do this, because I had observed that bees did not visit the flowers until they were open, *i.e.*, a day or two after they had been pollinated. The neglect of this precaution was justified by the event. With a single exception, all the crosses which "took" proved successful, as could be determined in the autumn of the year in which the cross was made in the case of G ♀ × Y ♂, and in the following autumn in the case of Y ♀ × G ♂. The exception referred to occurred in a cross between *Wen-dou* and a pure green—*Express*, viz., cross No. 71 (Table II). When the pod containing the F_1 cotyledons was opened, it was found to contain four yellow and *one green* seed. This was presumably due to the fact that the flower functioning as pistil-parent was operated on a little too late.

All the crosses between the pure yellow and extracted green are recorded in Table I. Those which gave no seed are indicated by being printed in *italics*. The date at which the crosses were made is written against the first of the crosses made on a given date. Table II is a list of the (successful) control crosses, to be described later on (p. 69).

Tables III to VI give the numbers of yellows and greens in individual families composing F_2 from the cross between the yellow and the extracted green, and a summary of all the families composing this F_2 is given after Table VI. Tables VII and VIII give details of F_2 from the control crosses. When there was any doubt as to the colour of a seed, it was recorded as doubtful. The number of such seeds, if they occur, in each family* is given in the column headed with a ?. The families of F_2 cotyledons from the cross between the pure yellow and the extracted green are given in two lots, in one of which (Tables III, IV, and V) it has been possible to classify the families according to the green and also according to the yellow parent forms from which they have sprung, and in the other of which (Table VI) this classification has not been possible, owing to an accident. This occurred during harvesting. The plant or plants bearing the F_2 families from cross 166 were accidentally incorporated in a bundle with the plants from some other cross. I discovered this by finding the peg with 166 on it, after the day's harvesting, rather deep in the ground (the top was just covered), as I was hoeing. So that, knowing the mistake had occurred that day, I was able to know in which bundles 166 had been incorporated. If I had kept a record of the number of seeds I sowed from each cross, I should have been able to discover the plants bearing the F_2 from cross 166 during the process of recording ; but I had not done this, in these particular sowings, partly because the ground had not been used for peas before (not in itself a sufficient justification), and partly because the seeds containing the F_1 cotyledons had been sown exactly 9 inches apart, so that if stray seeds *did* come up, they could not escape detection. (The rows were carefully examined during the process of weeding, which was done with great minuteness with the hand, the smallest seedlings being removed).† The families of F_2 cotyledons which cannot, as a result of this accident, be classified according to their yellow and green parent forms, have been given under a separate heading ; but it must be understood that one of them (which, there is no means of discovering) contained an additional family borne on one or more plants. I tried to identify it by the general look of its seeds, but although the F_2 cotyledons produced from the different types of yellows are often strikingly like their yellow grandparents, I did not succeed. The only drawback, however, which this accident entails is the impossibility of employing these families for the purpose of determining the percentage of greens from crosses from the several green and yellow parent forms. The total result from the F_2 families not classifiable by parent forms has been incorporated in the grand total; as also has a plant which is recorded as having been "accidentally left behind between 216 and 28 and not belonging to either." There were 161 yellow and 50 green seeds on it (see Summary after Table VI). Fortunately, all the plants from crosses between the pure yellow and extracted green were grown on a plot by themselves, so that there can be no question as to the parentage of a plant like this.

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* By "family" I mean, in the present case, the number of peas on a plant.

† I have reorganised the method of carrying out my experiments in such a way that no further mistakes of this kind can occur.

The Percentages of Greens in F_2 *produced from the several Yellow and Green Parent-forms.*

I have classified all the F_2 families, from the cross between the pure yellow and extracted green, into groups according to their yellow grandparents, and also according to their green grandparents, where this was possible (see previous section), and have determined the proportions of greens in each of these groups with a view to finding out whether the close approximation to 25 per cent., viz., 24·88 per cent., which was the proportion of greens in the whole population (including those families which could not be classified into groups), was also exhibited in each of these groups. The percentage of greens, calculated from the total numbers of yellows and green in the classifiable families is 24·82. Now it is quite conceivable that this total proportion may be compounded of widely divergent proportions, ranging perhaps from 10 to 40 per cent., and each, possibly, characteristic of families from particular yellow or green grandparents. In order to find out whether the deviations which might be expected to, and do, occur, are significant, or attributable to chance, it is necessary to calculate the Probable Error of the percentage. Half the deviations should fall inside and half outside the Probable Error in the *plus* or *minus* direction; and deviations greater than four times the Probable Error should not occur often. The Probable Error of the ratio, expressed as a percentage, of any part to a whole (in this case the ratio of the greens in a generation to the total number of seeds in that generation) is calculated by the following formula, which I have used here:—

$$\frac{100\left[0{\cdot}67449\times\sqrt{a\left(1-\frac{a}{x}\right)}\right]}{x},$$

in which x is the whole (or the total number of seeds) and a is the part (or the number of greens). The table on the next page shows that of the

11 groups, 5 exhibit a deviation in a − direction, and 6 in a + direction. Also that 7 of the deviations (478, 479, 481, 482, 493, 483, and 484) fall within the Probable Error and 4 outside, in only one of which is the deviation greater than thrice the Probable Error—viz., in 480 in which the actual deviation is a little over three and a-half times the Probable Error. The application of this test, therefore, justifies us in concluding that the deviations from 25 per cent. which occur in each of these 11 groups are attributable to chance.

	Catalogue number* of grand-parent.	Number of yellows.	Number of greens.	Per-centage of greens.	Actual deviation of per-centage.	Probable Error of per-centage.
Classification by yellow grandparents	477	14,081	4,532	24·35	−0·65	±0·212
	478	7,172	2,405	25·11	+0·11	±0·299
	479	20,761	6,951	25·08	+0·08	±0·176
	480	3,978	1,224	23·53	−1·47	±0·397
	481	2,777	942	25·33	+0·33	±0·481
	482	11,140	3,715	25·01	+0·01	±0·240
	492	14,157	4,658	24·76	−0·24	±0·212
	493	1,408	486	25·66	+0·66	±0·677
Totals	—	75,474	24,913	24·82	−0·18	±0·092
Classification by green grandparents	483	38,565	12,788	24·9	−0·1	±0·129
	484	17,150	5,719	25·01	+0·01	±0·193
	485	19,759	6,406	24·48	+0·52	±0·179
Totals	—	75,474	24,913			

* For signification of these, see pp. 62 and 63.

The totals have been added up and written under the grouping by yellow grandparents and under the grouping by green grandparents. But the percentage of greens, the actual deviation and Probable Error of the percentage calculated from these totals, have only been written once, *i.e.*, under the grouping by yellow grandparents.

A Special Case.

In the F_2 families raised from five of the crosses there occurred wrinkled as well as round seeds. All these occurred in crosses made with the extracted green No. 484, which is the only one of the three races of extracted greens used, from which, on the Mendelian theory, wrinkled seeds could arise; for, as the table on p. 62 shows, neither of the other green rounds used occurred in a family in which wrinkleds also occurred.

The nature of the mating enclosed in brackets in the pedigree on p. 61 and of its subsequent progeny is of the type

RR × DD
DR
RR DR DD

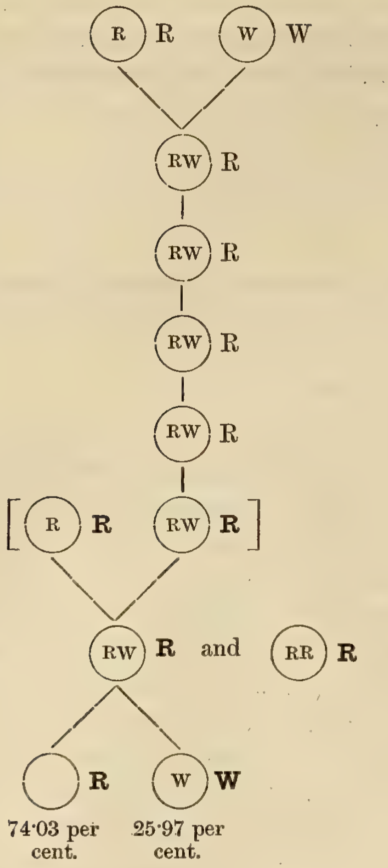
as far as the colour of the cotyledons is concerned. But with regard to the shape of the cotyledons, the nature of the mating, in those cases which gave wrinkleds in F_2, was of the type

DR × DD,

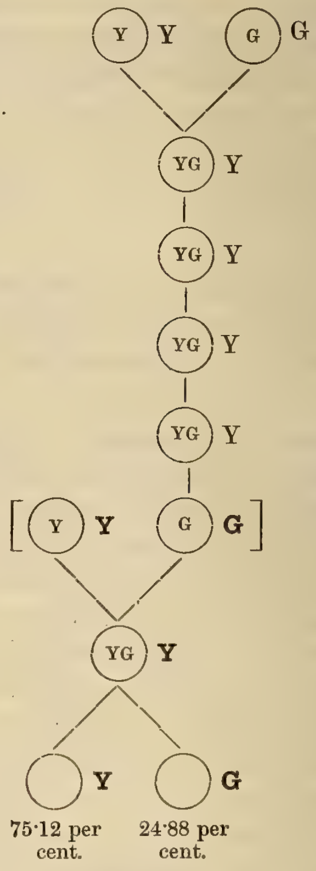
giving equal numbers of DR and DD individuals, the former of which, when allowed to self-fertilise, should produce rounds and wrinkleds in the proportion of 3 to 1. 14 families from 5 different crosses contained wrinkled seeds; in the case of 5 of these families I did not record the numbers of wrinkled, because I was not immediately alive to the interest of the case, and contented myself with recording the fact that both round and wrinkled did occur. In the remaining 9 families I recorded the numbers, and the totals were 2605 round and 914 wrinkled, which gives a percentage of 25·97 wrinkleds. Let us look for a moment at the ancestry, that is to say, the characters of the ancestors of this cross. The round hybrid, which gave rise to the F_2 in which this proportion of wrinkled occurred, was the result of the union of two *rounds*, one of them, the yellow, presumably a pure round, and the other, the extracted green, a hybrid round. This hybrid round was descended from an unbroken line of 4 "round" ancestors (each of them hybrids in the Mendelian sense); whilst behind that point half of the ancestors were round and half wrinkled. It will be convenient to display this ancestry in the form of a pedigree (Pedigree A), in which the crossing which gave rise to the F_2 in question is enclosed in a square bracket. Each ancestor is represented by a circle, and its character is written immediately to the right of the circle; the nature of the gametes borne by each ancestor being written within the circle.

Now let us write the pedigree on p. 61 out again (Pedigree B) in the same way as this, and compare the two (see p. 68).

The result obtained in F_2 from the experiment with cotyledon shape (Pedigree A) affords a more convincing *a posteriori* demonstration than that afforded by the experiment with cotyledon colour (Pedigree B), of the correctness of the course of leaving the soma out of account in the attempt to predict the result of a given mating. For, as is clearly seen by a


R R
W W
RW R
RW R
RW R
RW R
R R
RW R
RW R
and
RR R
R
W W
74·03 per cent.
25·97 per cent.

Pedigree A.


Y Y
G G
YG Y
YG Y
YG Y
YG Y
Y Y
G G
YG Y
Y
G
75·12 per cent.
24·88 per cent.

Pedigree B.

comparison of the two pedigrees, whereas in the case of cotyledon colour one of the parents of the hybrids, from the self-fertilisation of which the F_2 generation in question arose, bore the recessive character which reappeared in the proportion of 25 per cent. in that generation; in the case of cotyledon shape, *neither* of the parents exhibited the recessive character. Indeed, in the latter case the wrinkleds which appeared in F_2 had no ancestors bearing the wrinkled character for six generations behind them.

Subjoined are details of the families in which segregation into wrinkled and round occurred. Those in which I did not count the number of wrinkleds are simply marked DR. The families 158.1 to 6, and 175.1 to 4, fall into the unclassifiable group (see p. 64). They can, of course, be included in reckoning the percentage of greens in total of families in which segregation occurred, but not for calculating the relative numbers of families from DD and DR parents.

The actual deviation of the percentage of wrinkleds from expectation is +0·97; the Probable Error of this percentage is ±0·498: so that the actual deviation is just less than twice as great as the Probable Error, and is certainly not significant. Moreover, it is in the opposite direction to that which would afford any support to the theory of Ancestral Contributions.

	Number of family.	Nature of parent with regard to cotyledon colour.	Number of round.	Number of wrinkled.
Classifiable families	124. 1	DR	249	96
	124. 2	DR	219	64
	124. 3	DR	360	125
	165. 1	DR		
	165. 2	DD		
	165. 3	DD		
	165. 4	DR	393	142
	218. 1	DR	162	61
	218. 2	DR	408	147
	218. 3	DR	219	75
	218. 4	DD		
	218. 5	DD		
Unclassifiable families ...	158. 1	DD		
	158. 2	DR		
	158. 3	DR		
	158. 4	DR	297	103
	158. 5	DD		
	158. 6	DR	298	101
	175. 1	DR		
	175. 2	DD		
	175. 3	DR		
	175. 4	DD		
			2605	914

Percentage of greens .... 25·97
Probable Error of percentage ...... ±0·498

The number of families (falling into the categories classifiable by yellow and green grandparents) in which segregation occurred was 8; the number of families from the same parents in which segregation did not occur was 4. There should, on the Mendelian hypothesis, be equal numbers of the two (DR and DD give 50 per cent. DR, and 50 per cent. DD). But I do not think it desirable to base any conclusion on numbers so small as these.

Control Crosses.

The evidence on which the statement that the greens occur in the proportion of 25 per cent. in the F_2 generation from a cross between a pure yellow and a pure green pea is based, has been collected by Mr. Lock in his most useful paper on "The Present State of Knowledge of Heredity in

Pisum."* Subjoined is the table in which the details of this evidence are summarised by Mr. Lock. I give it in full because it embodies the result in F_2 from the cross made by Mr. Hurst, in F_5 from which the extracted greens which I used in my experiment occurred. The percentage of greens calculated from the total is 24·9. The actual deviation is less than the Probable Error.

Observer.	Yellow.	Green.	Green, per cent.
Mendel	6,022	2,001	24·9
Correns	1,394	453	24·5
Tschermak	3,580	1,190	24·9
Bateson	11,903	3,903	24·7
Hurst	1,310	445	25·4
Lock	1,438	514	26·2
	25,647	8,506	24·9

Besides the above, I have some evidence based on observations made by me which more strictly deserve to rank as control observations. This evidence is based on the result of crosses made between the identical yellow races used in the experiment, and, with the exception of the few crosses made in 1906, from the pollen from the identical crop of plants which were the yellow parents in the cross between the pure yellow and extracted (F_5) green (see Table II). The details of the F_2 families raised from these control crosses are given in Tables VII and VIII. The total number of yellows raised was 4015, and of greens 1394, the percentage of greens being 25·77. The Probable Error of the percentage is $\pm 0·401$, so that the actual deviation, being less than twice the Probable Error, is certainly not significant.

The expense of carrying out this experiment was defrayed by a grant from the Government Grant Committee of the Royal Society, and I take this opportunity of expressing my indebtedness to them. I also wish to express my thanks to Mr. Udny Yule and to Dr. E. Schuster for some preliminary statistical assistance, and to Mr. Charles Biddolph for clerical help.

Summary.

An experiment has been devised to test the truth of the theory of Ancestral Contributions: the results of the experiments prove:—

(*a*) That the phenomena of dominance, and, what is more important, of the segregation of characters in definite proportions, are independent of the ancestry (and of the geographical source) of the parent-forms mated.

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* 'Annals of the Royal Botanic Gardens, Peradeniya,' vol. 4, Part III.

(*b*) That the recessive character which reappears in F_5 is as pure as that borne by a pure race, as tested by the results obtained from its union with a pure dominant character.

(*c*) That there is nothing like Ancestral Contributions within the limits of a single unit-character.

(*d*) That in the attempt to predict the result of a given mating the somatic characters not only of the parents and of the ancestors* of the individuals mated, but of the individuals themselves,† may be entirely left out of account; and that the expectation based on a theory of the contents of the germ cells of the two individuals mated is fulfilled.

SYNOPSIS OF CONTENTS OF TABLES AND SUMMARIES.

Those tables (viz., II, VII, and VIII) which embody the result of the control observations (see p. 70) are printed in italics.

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* See Pedigree B on p. 68.

† See Pedigree A on p. 68.

Table I.—Matings between Pure Yellows and Extracted Greens in F_5. (See pp. 61—65).

Number of cross or crosses.	Number of ♀ parent.	Number of ♂ parent.	Date of making cross (all 1907).
20, *21*	485	493	VI.2
22, 23	492	485	VI.9
24, 25, 26, 27, 28	485	492	
75, 76, 77, *78*	483	477	VI.16
79, 80, 81, *82*	483	477	
83, 84, 85, 86, 87	483	492	
88, 89, *90*, 91	485	492	
108, 109, *110*, 111	477	483	
112	483	477	
113, 114, 115, 116	483	477	VI.22
117, *118*, 119, 120	483	477	
⎰121	483	479	
⎱*122*, 123, 124	484	479	
125, 126, 127, 128	485	479	
$\overbrace{129,\ 130}$, 131, 132	485	492	
143, 144, 145, *146*	483	479	VI.26
147, 148, 149, 150	483	479	
151, 152, 153, 154	483	479	
155, 156, *157*, 158	484	479	
159, *160*, 161, 162	483	492	
⎰*163*, 164	483	480	
⎱165, 166†	484	480	
167, *168*, *169*, 170	484	481	
171, 172, *173*, 174	484	477	
175, *176*, 177, 178	484	478	VI.29
179, 180, 181, 182	483	479	
183, 184, 185, 186	483	480	
$\overbrace{187,\ 188}$, 189, 190	485	492	
191, 192, $\overbrace{193,\ 194}$, 195, 196	484	479	
205, 206	483	477	VII.1
217, 218	484	493	
⎰$\overbrace{223,\ 224}$	485	482	VII.2
⎱225, *226*	484	482	
227, 228	478	484	
229, *230*	485	478	VII.6
231	484	478	
232, 233, *234*, 235	478	483	
236, 237, 238, 239	482	483	
⎰240	483	479	VII.7
⎱241, 242, 243	485	479	
263, *264*, 265, 266	482	483	
267, 268, *269*, 270	482	485	
271, 272, 273	485	494	
$\overbrace{274,\ 275}$, *276*, *277*	481	484	VII.14

* See p. 64.

NOTE.—The association of crosses (such as 129 and 130) by a horizontal bracket indicates that the two stigmas pollinated were in flowers on the same peduncle.

Table II.—Matings between Pure Yellows and Pure Greens. (See p. 70.)

	Number of cross or crosses.	Number of ♀ parent.	Number of ♂ parent.	Date of making cross.
1906	29	329	404	VI.10
	31, 32	323	404	
1907	40	477	491	VI.9
	44	477	491	
	69, 70, 71*	491	477	VI.10
	97, 98, 99	480	491	VI.16
	104, 105	491	477	
	200	491	479	VI.29
	204	491	477	
	208	491	479	VII.1
	210	491	477	
	244	491	479	VII.7
	250	491	477	
	252, 254, 255	491	478	
	256	491	480	
	258, 259	491	481	
	260, 261	491	482	

* See note on p. 64.

Note.—The significations of the numbers indicating the parents in the 1907 crosses will be found on p. 63. The following are the parents of the 1906 crosses (the first three on the list):—323 = "Express" (green round); 329 = Laxton's "Alpha" (green wrinkled); 404 is the 1906 sowing of the yellow round type which appears as 492 and 493 in the 1907 crosses (see p. 63).

Details of F_2 Families, produced from Crosses in Table I, classifiable by Yellow and Green Grandparents.

Table III.—Particulars of F_2 Families from Crosses made with Extracted Green No. 483.

Number of family.	Y.	G.	?	Number of family.	Y.	G.	?	Number of family.	Y.	G.	?
75. 1	146	52		77. 4	13	11		85. 1	74	26	
75. 2	82	20		77. 5	103	37	3	85. 2	73	28	
75. 3	172	55	2	79. 1	107	31		85. 3	138	43	2
75. 4	142	42	1	79. 2	96	30		86. 1	203	54	
75. 5	149	50	4	79. 3	32	10		86. 2	157	58	
75. 6	160	63	1	79. 4	129	41		86. 3	103	48	
75. 7	24	14		79. 5	172	47		86. 4	85	31	
76. 1	196	68	1	81. 1	38	22		87. 1	38	9	
76. 2	133	41		81. 2	153	54		87. 2	73	27	
76. 3	64	27		81. 3	190	52		87. 3	71	27	1
76. 4	118	36		81. 4	152	52		87. 4	122	44	1
76. 5	283	95	2	81. 5	141	44	2	87. 5	173	57	
76. 6	100	31		81. 6	63	26		87. 6	83	32	
76. 7	114	34	1	83. 1	95	36		108. 1	360	114	2
76. 8	158	48		84. 1	31	18		108. 2	343	115	
77. 1	8	2		84. 2	67	23		109. 1	41	15	
77. 2	52	12		84. 3	50	17		109. 2	174	58	3
77. 3	69	21		84. 4	122	38		109. 3	36	16	

Table III—*continued.*

Number of family.	Y.	G.	?	Number of family.	Y.	G.	?	Number of family.	Y.	G.	?
109. 4	33	13		144. 1	40	12		186. 2	300	97	
109. 5	66	15		144. 2	20	9		186. 3	216	57	1
109. 6	41	10		144. 3	249	77	1	195. 1	184	58	
112. 1	18	15		148. 1	361	103	1	195. 2	231	85	
112. 2	343	93		148. 2	266	98	3	195. 3	181	65	
112. 3	241	77		148. 3	19	5		195. 4	169	63	
112. 4	163	43		148. 4	175	78		206. 1	346	115	3
112. 5	129	39		152. 1	349	120	5	206. 2	173	68	
113. 1	132	47		152. 2	286	95	6	206. 3	608	201	6
113. 2	149	56		152. 3	194	58	3	206. 4	103	39	
113. 3	75	26	4	153. 1	118	48	4	232. 1	528	180	1
113. 4	115	45		153. 2	56	20		232. 2	229	84	2
113. 5	124	40		153. 3	53	8		232. 3	241	84	2
113. br.*	38	15		153. 4	97	27	3	233. 1	224	86	
114. 1	86	22	1	154. 1	270	93	3	233. 2	224	76	
114. 2	172	68		154. 2	158	46		233. 3	164	64	
114. 3	159	45	2	154. 3	190	66	3	233. 4	221	79	1
114. 4	279	84	4	154. 4	265	80		233. 5	79	24	
114. 5	119	52		154. 5	307	96		233. 6	201	75	
114. 6	130	45	1	159. 1	91	28		233. 7	59	18	
114. 7	169	44		159. 2	138	55		233. 8	76	39	1
115. 1	123	36		159. 3	169	42		235. 1	173	57	
115. 2	135	54		159. 4	265	83		235. 2	203	57	
115. 3	176	58	3	161. 1	360	103	2	235. 3	99	26	
116. 1	154	50		161. 2	176	69	3	235. 4	127	47	
117. 1	376	110		161. 3	290	78	8	235. 5	206	61	6
117. 2	257	97	2	162. 1	156	56		235. 6	139	36	
117. 3	305	98	2	162. 2	167	65	5	237. 1	483	200	1
119. 1	246	72	2	162. 3	295	110	2	237. 2	364	125	2
119. 2	150	43		162. 4	155	48		238. 1	378	127	1
119. 3	72	22	1	162. 5	212	71	1	238. 2	430	134	3
119. 4	30	6		162. 6	159	66	1	240. 1	321	102	4
119. 5	317	93	6	164. 1	150	56	2	240. 2	299	98	
119. 6	375	127	3	164. 2	286	68	4	240. 3	127	37	
120. 1	161	53		164. 3	151	45		240. 4	263	110	
120. 2	87	32	1	164. 4	194	59	2	240. 5	267	76	
120. 3	127	46		164. 5	55	15		265. 1	165	59	
120. 4	121	25		164. 6	247	81	2	265. 2	291	74	
120. 5	209	64	5	164. 7	222	62	6	265. 3	408	123	5
120. 6	250	68	1	164. 8	263	91	10	265. 4	449	138	3
121. 1	198	65		164. 9	119	35	8	265. 5	296	98	
121. 2	288	97		179. 1	334	112	3	265. 6	204	67	
121. 3	253	97	1	179. 2	481	157	8	265. 7	429	184	2
121. 4	244	79		179. 3	285	108		266. 1	355	128	3
121. 5	324	94	1	179. 4	442	163		266. 2	403	116	6
121. 6	379	131		179. 5	391	152	1	266. 3	25	6	
121. 7	45	10		179. 6	504	181		266. 4	465	152	1
121. 8	362	109	2	179. 7	387	142	1	266. 5	202	64	1
143. 1	191	56		186. 1	234	72		266. 6	379	129	3

* A branch.

Table IV.—Particulars of F_2 Families from Crosses made with Extracted Green No. 484.

Number of family.	Y.	G.	?	Number of family.	Y.	G.	?	Number of family.	Y.	G.	?
124. 1	260	85	4	191. 2	253	102	1	225. 2	134	44	
124. 2	205	78	2	191. 3	310	103	2	225. 3	339	108	1
124. 3	378	107	1	192. 1	269	89	2	227. 1	132	51	2
156. 1	279	96		192. 2	311	109	1	228. 1	207	58	5
156. 2	419	125		192. 3	497	175		228. 2	100	37	1
156. 3	311	90		192. 4	408	144	1	228. 3	165	52	1
165. 1	546	168	3	193. 1	72	22		228. 4	207	54	
165. 2	226	79	6	193. 2	270	87	1	228. 5	206	63	1
165. 3	353	120		193. 3	318	126	2	228. 6	271	85	5
165. 4	416	119	2	194. 1	215	79	1	228. 7	245	89	1
172. 1	436	112		194. 2	108	37		231. 1	133	40	
172. 2	273	84		194. 3	166	48	3	231. 2	108	36	
172. 3	81	17	1	194. 4	34	7		231. 3	378	151	1
172. 4	265	92	1	194. 5	168	65	6	274. 1	439	141	3
172. 5	316	99	1	194. 6	125	34	1	274. 2	334	88	
174. 1	278	97		218. 1	165	58	10	275. 1	326	108	
174. 2	237	74		218. 2	422	133	2	275. 2	342	127	
178. 1	239	98	2	218. 3	213	81	3	275. 3	179	73	
178. 2	150	48	2	218. 4	514	178	4	275. 4	399	153	1
178. 3	339	114	4	218. 5	94	36		275. 5	375	127	
178. 4	176	58	1	225. 1	380	154	1	275. 6	383	125	
191. 1	253	82	2								

Table V.—Particulars of F_2 Families from Crosses made with Extracted Green No. 485.

Number of family.	Y.	G.	?	Number of family.	Y.	G.	?	Number of family.	Y.	G.	?
22. 1	86	35		91. 3	388	128	1	188. 1	165	45	
22. 2	28	11		91. 4	271	86	1	188. 2	172	59	
22. 3	118	43		125. 1	83	28	1	188. 3	192	72	
22. 4	138	30		126. 1	72	31		188. 4	195	64	
22. 5	205	65	1	127. 1	371	112	3	190. 1	14	1	2
22. 6	220	59	1	127. 2	354	114	1	190. 2	25	4	
22. 7	206	71		127. 3	257	98		190. 3	82	19	3
23. 1	33	12		127. 4	273	94	1	223. 1	255	83	2
23. 2	174	50		127. 5	370	123		223. 2	434	142	6
23. 3	78	21		127. 6	257	88		223. 3	264	82	3
23. 4	230	64		128. 1	262	77		223. 4	411	128	4
23. 5	112	44		128. 2	394	137		229. 1	270	88	
24. 1	213	78		128. 3	297	100		229. 2	202	56	
24. 2	206	66	1	128. 4	270	75	2	229. 3	137	37	
25. 1	129	35		128. 5	328	113		229. 4	314	97	
25. 2	97	42		128. 6	337	107		242. 1	129	43	
26. 1	282	109		128. br.	51	16		242. 2	26	4	
26. 2	155	54	1	129. 1	216	70		242. 3	113	31	1
26. 3	237	87	1	129. 2	275	89	5	242. 4	265	84	
26. 4	123	52		130. 1	295	78		267. 1	85	28	
28. 1	153	45	2	130. 2	89	35	2	267. 2	412	122	1
28. 2	96	38		130. 3	269	88	2	267. 3	212	73	1
88. 1	123	40		130. 4	65	10		267. 4	234	84	1
88. 2	34	10		130. 5	227	80	3	267. 5	179	58	
88. 3	255	56		131. 1	268	87	3	267. 6	267	101	
88. 4	209	55		131. 2	341	122	6	268. 1	29	5	1
88. 5	123	36	1	132. 1	153	45		268. 2	79	24	5
88. 6	91	20		132. 2	71	27	1	270. 1	407	118	3
89. 1	186	51	2	132. 3	157	52	1	270. 2	133	46	
89. 2	149	53	1	132. 4	225	75	11	270. 3	414	155	5
89. 3	33	17		132. 5	260	88	7	270. 4	320	107	4
89. 4	156	55	1	187. 1	88	33		270. 5	22	10	
91. 1	197	64		187. 2	187	71	4	270. 6	404	119	2
91. 2	201	72									

Table VI.—Particulars of F_2 Families, from Crosses between Pure Yellow and Extracted (F_5) Greens, not Classifiable by Yellow and Green Grandparents.

Number of family.	Y.	G.	?	Number of family.	Y.	G.	?	Number of family.	Y.	G.	?
27. 1	194	68	1	170. 3				189. 3	253	67	1
27. 2	141	49		and	382	123	1	189. 4	200	74	
80. 1	169	56		170. 4*				189. 5	146	57	4
80. 2	133	35	1	170. 5	291	91	1	189. 6	21	9	
80. 3	111	29		175. 1	75	26		189. 7	247	74	2
111. 1	237	70	3	175. 2	153	49		196. 1	459	170	5
123. 1	518	175	7	175. 3	138	34		196. 2	271	91	6
123. 2	216	74	1	175. 4	263	99		196. 3	194	50	1
123. 3	333	126	3	177. 1	205	76		196. 4	270	91	3
123. 4	295	106	7	177. 2	248	70		196. 5	122	38	
123. 5	386	124	2	177. 3	251	92		196. 6	150	49	1
123. br.	36	16		180. 1	178	53	1	196. br.	12	1	1
145. 1	228	71	3	180. 2	325	109	3	205. 1	117	34	
145. 2	132	35	2	180. 3	283	106	3	205. 2	146	37	1
145. 3	147	59		180. 4	264	89		205. 3	261	97	
145. 4	174	57		180. 5	226	64	2	205. 4	335	118	1
145. 5	214	69	5	181. 1	134	43	3	205. 5	265	71	
145. 6	311	121		181. 2	457	163	5	224. 1	463	144	3
145. 7	82	27		182. 1	212	61	3	224. 2	71	27	
145. 8	260	106	1	182. 2	292	114	4	224. 3	455	143	1
149. 1	251	87	7	182. 3	290	92		224. 4	447	186	
149. 2	490	165	12	182. 4	126	51		239. 1	232	73	1
149. 3	192	50	5	182. 5	278	79	4	239. 2	105	24	
149. 4	326	109	5	182. 6	351	127		239. 3	490	167	
149. 5	176	42	6	182. 7	406	128	3	239. 4	323	96	1
149. 6	309	106	11	183. 1	233	70	2	239. 5	136	42	
150. 1	225	75	1	183. 2	164	62		239. br.	73	26	
155. 1	318	110		183. 3	158	46	1	241. 1	388	126	2
155. 2	516	201		183. 4	16	7		241. 2	312	106	1
158. 1	282	109		183. 5	117	39		241. 3	119	39	
158. 2	132	47		183. 6	234	74	3	241. br.	25	9	
158. 3	146	40		184. 1	254	89	2	243. 1	186	70	1
158. 4	296	104		184. 2	346	108	1	243. 2	153	53	
158. 5	268	88		185. 1	283	94	2	243. 3	189	66	
158. 6	304	95		185. 2	350	106		243. 4	204	83	
167. 1	359	128		185. 3	309	87		271. 1	192	68	
167. 2	270	93		185. 4				271. 2	243	69	2
167. 3	519	203	1	and	560	185	2	271. 3	186	73	
167. 4	314	86		185. 5*				272. 1	269	96	2
170. 1	416	133	2	185. 6	270	112		272. 2	259	66	1
170. 2	138	48	1	189. 1	164	52		272. 3	133	57	
				189. 2	19	7		273. 1	419	123	

* By an accident the two families bracketed were recorded together.

NOTE.—A glance at Tables III to VI (Experimental F_2) on the one hand, and at VII and VIII (Control F_2) on the other is, alone, sufficient to reveal an enormous difference in the average size of the family, *i.e.*, in the average number of seeds on a plant, in these two groups. The superiority of the former group is due to the fact that the seeds which gave rise to the plants recorded in it were sown 9 inches apart, that the plot on which the plants grew was dug two spits deep, a heavy dressing of stable manure being thrown on to the top of the lower spit; whereas the plot on which the Control F_2 families (those recorded in Tables VII and VIII) was only dug one spit deep and received no manure, except a dressing of superphosphate of lime and steamed bone-flour which was also given to the other plot. I have evidence, as yet unpublished, that the proportion of yellow and green seeds is *not* affected by the nutrition of the plant.

Summary of F_2 Families from Crosses between Pure Yellows and Extracted (F_5) Greens.

	Yellow.	Green.
Table III	38,565	12,788
„ IV	17,150	5,719
„ V	19,759	6,406
„ VI	29,410	9,829
Family referred to on p. 65	161	50
Totals	105,045	34,792

Percentage of green	24·88
Actual deviation	−0·12
Probable Error of percentage	±0·078

Table VII.—Particulars of F_2 Families from Control Crosses made in 1906.

Number of family.	Y.	G.	?	Number of family.	Y.	G.	?	Number of family.	Y.	G.	?
29. 1	108	29		31. 4	103	33		32. 4	46	18	
29. 2	95	22		32. 1	22	9		32. 5	72	25	
31. 1	73	26		32. 2	60	19		32. 6	70	23	
31. 2	227	78		32. 3	76	27		32. 7	110	31	
31. 3	84	19									

NOTE.—For details of parentage, see Table II.

Table VIII.—Particulars of F_2 Families from Control Crosses made in 1907 between the Pure Yellow Types used in the Experiment and "Express" a Pure Green (Round) Type.

Number of family.	Y.	G.	?	Number of family.	Y.	G.	?	Number of family.	Y.	G.	?
40. 1	24	12		105. 1	12	2		255. 2	32	19	
40. 2	52	13		105. 2	12	3		255. 3	64	19	
40. 3	49	17		105. 3	38	14		255. 4	27	7	
40. 4	11	4		105. 4	40	12		255. 5	31	10	
40. 5	49	20		105. 5	18	8		255. 6	20	14	
44. 1	12	4		105. 6	24	6		255. 7	45	18	
69. 1	15	6		200. 1	22	7		256. 1	34	9	
69. 2	9	6		204. 1	44	14		256. 2	61	16	1
69. 3	23	8		204. 2	28	16	2	256. 3	33	22	
69. 4	2	2		208. 1	23	7		256. 4	37	11	2
69. 5	30	11		208. 2	18	6		256. 5	52	20	1
70. 1	23	7		208. 3	48	16		258. 1	34	22	
70. 2	7	1		210. 1	15	8		258. 2	15	8	
70. 3	41	16		210. 2	32	6		258. 3	7	4	
71. 1	2	1		244. 1	39	13	2	258. 4	43	15	
71. 2	45	14		244. 2	7	5		259. 1	3	2	
97. 1	1	—	1	244. 3	64	24		259. 2	8	1	
97. 2	24	10		250. 1	8	4		259. 3	14	2	
97. 3	52	19		250. 2	26	11		259. 4	35	11	
98. 1	40	9		250. 3	34	18		260. 1	87	27	
98. 2	51	14	1	250. 4	17	8		260. 2	21	6	
99. 1	86	39		250. 5	5	2		260. 3	26	7	
104. 1	15	10		252. 1	43	11		260. 4	69	20	
104. 2	29	12		252. 2	29	19		260. 5	24	8	
104. 3	58	20		254. 1	25	8		261. 1	7	2	
104. 4	9	2		254. 2	31	16		261. 2	104	32	
104. 5	29	11		254. 3	25	12		261. 3	74	22	
104. 6	18	7		254. 4	88	31		261. 4	51	14	
104. 7	28	13		255. 1	53	15		261. 5	109	37	

NOTE.—For details of parentage, see Table II.

Summary of F_2 Families from Control Crosses.

	Yellow.	Green.
Table VII	1146	359
,, VIII	2869	1035
Totals	4015	1394

Percentage of green	25·77
Actual deviation	+0·77
Probable Error of percentage	±0·401

The Action of the Venom of Sepedon hæmachates *of South Africa.*

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(From the Pharmacology Laboratory of the University of Edinburgh.)

(Abstract.)

The venom used was an extract from the dried venom glands of the *Sepedon hæmachates.* Its minimum lethal dose by subcutaneous injection per kilogramme was found to be: for the frog, 0·0012 gramme; for the rabbit, 0·001 gramme; for the rat, 0·0016 gramme; for the cat, 0·015 gramme; for the pigeon, 0·0033 gramme; and, by intravenous injection, for the rabbit, 0·00055 gramme.

In the case of all these animals, the venom primarily and with greatest intensity affects the respiration. Respiratory paralysis is the cause of death in mammals and in birds; in frogs, the respiratory movements are early paralysed, but death occurs after several days from gradual failure of the circulation. Other conspicuous effects produced by lethal doses in mammals are drowsiness, ataxia, impairment of reflexes, and fall of temperature. In frogs, the venom produces diminution of reflex excitability, motor paralysis, and progressive increase in weight due to œdema.

In warm-blooded animals, the venom has a marked enfeebling action on the brain and spinal cord, which is only slightly, if at all, produced on the motor nerve ends. In frogs, however, motor paralysis is due to a paralysing action both on the central nervous system and on the motor nerve ends, the former action being characteristic especially of large doses, the latter being more pronounced in the late stages of poisoning with smaller doses.

The venom has, comparatively with its action on nerve structures, a very slight action on skeletal muscle.

From the point of view of lethality, the effects of the venom on the circulation are of minor importance compared with those on the respiration. Perfused through the frog's heart, strong solutions of Sepedon venom bring about an increase of the rate, followed by arrest of the heart in systole; and weaker solutions slow the heart and arrest it in diastole. The latter effect is the only one manifested after injection of even 10 times the minimum lethal dose.

Strong solutions slightly constrict the frog's blood-vessels when perfused through them.

In rabbits, the venom injected intravenously causes a slight fall of blood-pressure. This is soon recovered from, and thereafter the blood-pressure rises and remains high till the end of life. The transient fall of blood-pressure is probably mainly due to a weakening of the heart's contraction. When pronounced embarrassment of the respiration comes on, the blood-pressure rises above the normal level. This is mainly due to stimulation of the vaso-motor centre by the venous condition of the blood, the heart being at the same time slowed through stimulation of the vagus. The venom also slightly slows the heart by a direct action on it, and the direct but slight constriction of the vessels may be a contributing factor in maintaining the level of the blood-pressure.

In the course of poisoning in frogs, the lymph hearts are paralysed tardily, but long before the blood heart.

Sepedon venom has little action on the blood. It does not definitely affect the coagulability, and neither hæmorrhages nor intravascular clotting are found *post mortem*. Hæmolysis is not found *in vivo*.

Respiratory failure in mammals is due to paralysis of the respiratory centre, the excitability of the phrenic nerve-ends being practically unimpaired.

Non-lethal doses of Sepedon venom cause a rise of temperature; lethal doses cause a fall of temperature, with sometimes an initial rise.

The Selective Permeability of the Coverings of the Seeds of Hordeum vulgare.

By Adrian J. Brown, Professor of Brewing in the University of Birmingham

(Communicated by Prof. H. E. Armstrong, F.R.S. Received January 23,—
Read January 28, 1909.)

The seeds of the variety of barley known as *Hordeum vulgare* var. *cærulescens* owe their colour to the presence of a blue pigment in the aleurone cells; this pigment, like litmus, is turned red by acids. Such seeds, when immersed in a dilute solution of sulphuric acid, if their coverings are damaged, soon turn pink in colour, which is a proof that acid diffuses into the endosperm; sound seeds, on the other hand, although they imbibe water freely from the solution, becoming soft and swollen, retain their colour, showing that the covering has the property of resisting the passage of the acid, whilst it allows water to diffuse freely into the interior of the grain. So much is this the case that a dilute solution of sulphuric acid may be concentrated by steeping barley in it. Thus in an experiment with a solution containing 4·9 grammes of acid per 100 c.c. it was found that the concentration of the acid was increased to 7·6 grammes per 100 c.c. In another case, in which the weight of water absorbed was ascertained, it was observed that the concentration effected was in direct proportion to the amount of water absorbed by the seeds.

Having made the discovery of so remarkable a "semi-permeable" membrane, I have endeavoured to ascertain its behaviour towards substances generally. In my earlier experiments, of which an account has been given elsewhere,* it was found that sulphuric acid could not penetrate into the grain, not only from volume normal solutions, but also from solutions containing 9, 18, or even 36 grammes of acid per 100 c.c. In the case of the seeds immersed in the strongest acid, however, the interior remained dry, presumably because the power of the seed contents of imbibing water was insufficient to overcome the osmotic pressure of the liquid.

The vitality of the embryos was not destroyed by steeping the seeds in the acid solutions; when placed under suitable conditions they all germinated.†

When the blue seeds were immersed in a volume normal solution of hydrogen chloride, the colour remained unaltered, showing that there is no diffusion of acid into the grain.

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* 'Annals of Botany,' vol. 21, p. 79, 1907.

† Recent observations show that the barley corn displays a most remarkable power of withstanding the action of sulphuric acid. A number of blue corns, *i.e.*, those containing the neutral indicator for acid, were steeped in a volume normal solution of sulphuric acid during 48 hours, those corns which showed traces of red after this treatment being rejected

Solutions of caustic soda containing 1 per cent. or more of alkali disintegrate the seed covering; this resists the action of the alkali, however, if the liquid contain only ½ per cent., and after steeping seeds during several days in the solution, although water diffuses into the grain, no alkali enters.

Salts such as cupric sulphate, ferrous sulphate, potassium chromate, and silver nitrate were all found to be impenetrant substances.

Up to this point, it appeared that the covering of the seeds was a perfect "semi-permeable" membrane. Using iodine dissolved in a solution of potassium iodide, however, observations were made which indicated that the membrane possessed the power of selection—iodine was found to pass slowly into the seed until after several days it permeated the whole of the starchy endosperm, staining it a deep blue colour. That this result was not a consequence of the destruction of the membrane was proved by steeping seeds thus impregnated with iodine in a solution of sodium thiosulphate. So long as the seed coverings remained intact the iodine was unaffected, but when the coverings were ruptured the thiosulphate diffused rapidly into the seeds decolorising the iodine.

At this point my earlier studies were directed to an investigation of the nature and position of the particular covering of the seed of *H. vulgare*, which acted as the "semi-permeable" membrane. The experiments already described demonstrate that the embryo and endosperm of the seeds are enclosed within an envelope through which water and iodine diffuse readily, but through which salts and strong acids do not diffuse. As there appeared to be no recognised instance in the vegetable kingdom of a membrane other than one composed of living protoplasm possessing marked "semi-permeable" properties, it was obviously desirable to ascertain if the selective permeability of the seed-coverings were a function of the living tissue. From the

as faulty. Subsequently, the corns which remained blue were steeped continuously in the acid, and observed from time to time, with the following results :—

Time of steeping.	Percentage of corns remaining blue.
3 days	100
5 ,,	99
7 ,,	95
10 ,,	89
14 ,,	74
19 ,,	25
24 ,,	1

first this appeared to be very improbable, as the property was exhibited by the seeds in the presence of highly toxic acids and salts, which could hardly fail to arrest its vital activity if brought into contact with protoplasm. Experiments made with seeds which have been immersed in boiling water during varying periods extending to one hour have afforded conclusive evidence that the semi-permeability of the seed-coverings is not a function of living protoplasm.

Histological study of the seed-coverings indicates that their selective power is confined to the testa, and probably to that portion which is derived from the epidermis of the nucellus during the development of the seed.

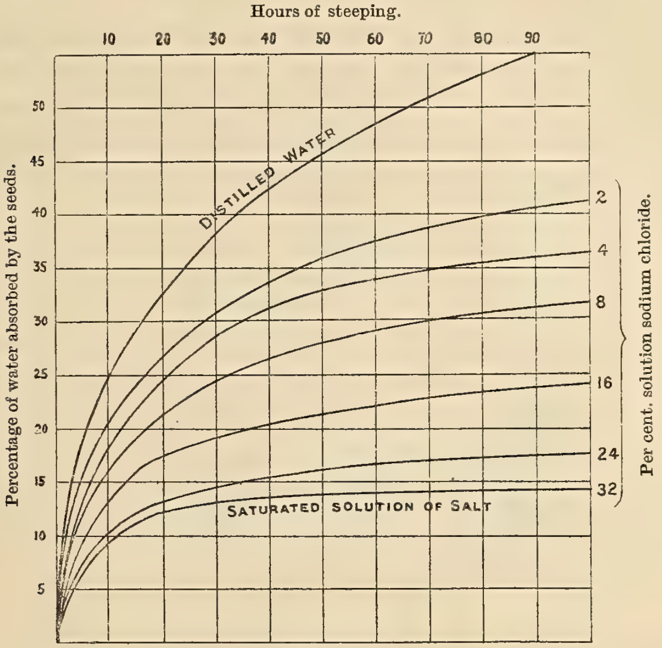
It appeared to be very desirable that the coverings should be removed and their behaviour studied apart from that of the seed contents. Although this has been attempted, the many experimental difficulties met with have not been satisfactorily overcome, and hitherto no other means of investigating their activity has been found than the somewhat unsatisfactory one of experimenting with whole seeds.

Behaviour of the Seed of H. vulgare *as a Semi-permeable System possessing a strong affinity for Water.*

When sound seeds of *H. vulgare* are immersed in an aqueous solution containing a substance which cannot pass through the outer semi-permeable membrane, and water is absorbed, presumably the seed contents enter into competition with the solute for the water. From this point of view, it appeared to be important to ascertain to what extent water would be absorbed from various solutions, and to compare the amounts with that absorbed when the seeds were placed in water alone.

The method adopted in all the experiments to be referred to was as follows: —A known weight of selected air-dried seeds, usually about 5 grammes, having been steeped in the solution under examination during some desired period, the seeds were separated from the liquid by means of a small wire-gauze strainer. After draining for a few minutes they were placed between the folds of a soft, dry cloth and were rubbed gently to remove as much of the adherent liquid as possible; they were then weighed. It is obvious that such a method cannot afford absolute values; but experience shows that concordant results may be arrived at without difficulty if all operations in connection with draining and drying the seed are carried out in as constant a manner as possible. The water absorbed is expressed as a percentage weight calculated on the original dry weight of the seeds.

The results of a series of observations with solutions of common salt containing from 2 to 32 grammes of salt per 100 grammes of solution are recorded graphically in the accompanying diagram.



It will be noticed that the amount of water absorbed by the seeds when equilibrium is established is less the more concentrated the solution, as it varies from about 14 per cent. in the case of a saturated solution to about 41 per cent. in the case of a solution containing only 2 per cent. of salt; this latter amount is much below that absorbed when the seed is steeped in water alone (over 70 per cent.).

It should be pointed out that the determinations on which the curves are based are affected by an unavoidable error due to the manner in which the values are arrived at. Some allowance should be made for the amount of water absorbed by the outer covering of the seeds. It is not possible to evaluate this amount very closely; apparently, however, it may be taken as equal to about 8 per cent. of the original weight of the dry seed. Making the extreme assumption that the amount absorbed by the outer covering is independent of the concentration of the solution, the quantity absorbed by the starchy contents of the seed from a saturated solution of salt is only about (14 — 8 =) 6 per cent. calculated on the weight of the dry seed. It appears, therefore, that the power of the seed contents of attracting water from a saturated solution of salt exceeds the osmotic attraction of the latter to only a slight extent; as the "osmotic pressure" of a saturated solution of sodium chloride is about 125 atmospheres, the power with which water is absorbed by the seed contents probably corresponds to a pressure somewhat in excess of this value.

Table I contains the results of a further series of determinations, made with a variety of solutes, in which seeds of *H. vulgare* were steeped in the liquid until equilibrium was established; in all cases the solutions were of volume normal strength, *i.e.*, they contained a molecular proportion in grammes of the dissolved substance per litre of solution.

Table I.

Solute.	Percentage of water absorbed.
$NaCl$	37·4
$NaNO_3$	37·9
KCl	37·1
KNO_3	40·5
$CuSO_4$	41·7
H_2SO_4	37·8
Tartaric acid	42·2
Cane-sugar	39·3
Water (control)	74·3

Although the various solutes appear to regulate the diffusion of water into the seeds in a very similar manner, minor differences are observable; thus, contrasting sodium chloride with potassium nitrate, there is an excess of 3 per cent. in the amount of water absorbed from the solution of the latter. This small excess was at first regarded as an experimental error, the method being open to suspicion when small differences are concerned; but as all experiments made subsequently with the same salts have consistently afforded similar results, there can be little doubt that the small departure is real. Small differences are also noticeable between the values for cupric sulphate and tartaric acid in comparison with that afforded by sodium chloride.

As the amount of water present in a *volume normal* solution varies from substance to substance, experiments were now made with *weight normal* solutions equivalent in strength, prepared by dissolving the solute always in the proportion of 1 gramme molecular proportion to 1000 grammes (55·5 molecular proportions) of water. The results obtained are recorded in Table II, the values in the last column being those observed when equilibrium was established. Although they are in close general agreement with those obtained on using volume normal solutions, small specific differences between the salts become apparent, the potassium salt being less

Table II.

Solute.	Percentage of water absorbed after steeping the seeds during—				
	2 days.	4 days.	6 days.	8 days.	11 days.
KCl	31·2	36·5	36·9	37·3	38·4
NaCl	30·5	34·2	35·7	37·3	37·2
NH_4Cl	31·7	34·6	36·4	36·6	37·4
KNO_3	34·1	38·7	40·5	41·1	41·6
$NaNO_3$	32·5	36·3	37·5	38·7	38·5
NH_4NO_3	32·3	36·1	38·3	38·4	38·9
Water (control)	43·1	55·6	64·1	68·3	70·0

active in the case both of the chloride and nitrate than either the sodium or ammonium salt, which behave alike.

In Table III are recorded the results of a direct comparison of the behaviour of the seeds towards dextrose and cane sugar in comparison with sodium chloride, in weight normal solutions. It will be seen that, although distinctly less active than salt, these two substances both inhibit the absorption of water to a very marked extent, being about equally effective.

Table III.

Solute.	Percentage of water absorbed in—				
	2 days.	4 days.	6 days.	7 days.	11 days.
Cane-sugar	29·5	34·3	36·2	36·9	38·4
Dextrose	30·2	35·8	38·1	38·3	39·8
NaCl	28·1	31·9	34·2	34·4	35·5

Selective Permeability of the Seed-coverings of H. vulgare.

Mercuric Salts.—When barley seeds are steeped in a 3-per-cent. solution of mercuric chloride in water, the salt may be detected within the seed covering after a few hours; after two or three days it is usually found diffused throughout the whole of the interior portions of the seeds.

At first it seemed possible that the passage of the salt into the seed ought not to be regarded as proof of a selective property of the seed-covering, but perhaps merely as an indication that the action of the salt destroyed their semi-permeable character. Experiment, however, has proved conclusively that this is not the case. When seeds which have been steeped in a solution of mercuric chloride during several days and then dried were steeped in a normal solution of sulphuric acid, it was found that the coverings still retained their original power of resisting the diffusion of sulphuric acid while permitting the diffusion of water into the seed.

Still more conclusive evidence of the possession by the seed-coverings of a differentiating power was furnished by an experiment in which seeds were steeped in a mixture of half a volume of a saturated solution of mercuric chloride with half a volume of normal sulphuric acid. After three days' steeping in this solution the mercuric salt was found to be diffused throughout the contents of the seed; even after five days' steeping, however, no trace of sulphuric acid could be found within the seed-coverings. The seeds of *H. vulgare* therefore possess the very remarkable property of absorbing mercuric chloride and rejecting sulphuric acid when steeped in a solution in which both are present. The exhibition of this property by the seeds appears to be of very special interest from a physiological point of view.

To ascertain their behaviour towards mercury salts generally, seeds were steeped in solutions of mercuric chloride, cyanide, nitrate and sulphate of equimolecular strength. The cyanide diffused as readily as the chloride into the seeds, but after several days no trace of mercuric salt could be recognised in those placed in the solutions of nitrate and sulphate. Moreover, the amount of water absorbed by seeds from solutions of mercuric chloride and cyanide did not differ from that taken up from water alone.

It should be noticed in passing that chloride and cyanide of mercury—which diffuse through the seed-coverings—are commonly regarded as but very slightly dissociated in aqueous solution, whilst mercuric sulphate and nitrate—which cannot penetrate the membrane—are salts which are supposed to be freely dissociated.

Cadmium Salts.—On steeping seeds of *H. vulgare* in volume normal solutions of cadmium iodide, chloride, and sulphate until equilibrium was established, the following results were obtained:—

Table IV.

Solute.	Percentage of water absorbed.
CdI_2	54·2
$CdCl_2$	46·3
$CdSO_4$	46·0
NaCl (control)	39·8

It will be noticed that the gain in weight in presence of the iodide is markedly greater than in presence of the chloride or sulphate, but that none of the salts has so considerable an effect as sodium chloride.

In the case of the seeds steeped in the solution of cadmium iodide—a salt which is supposed to be very slightly dissociated when dissolved in water—small quantities of the salt diffused through the coverings; cadmium was not detected, however, in the seeds which had been steeped in solutions of either the chloride or the sulphate, which are supposed to undergo dissociation to a somewhat limited extent, although to a greater extent than the iodide.

Acetic and other Weak Acids.—On searching for compounds capable of passing through the seed-coverings, when it was found that acetic acid possesses the property, it at first seemed probable that the acid might be capable of destroying the semi-permeable layer of the seed. Seeds were therefore steeped in solutions containing both acetic and sulphuric acids or acetic acid and cupric sulphate; in both cases only acetic acid and water diffused through the coverings.

Water also passes freely together with the acid into the seeds. The results of experiments with a volume normal solution of the acetic acid are recorded in Table V; it will be noticed that the acid has but a slight influence in diminishing the amount of water which is absorbed when equilibrium is established.

Table V.

Solute.	Percentage of water absorbed.
Acetic acid	73 ·8
Water (control)	78 ·2
Sodium acetate (control)	39 ·8

On examining the behaviour of the seeds towards organic acids other than acetic, it was found that formic, propionic and butyric acids also enter the seed system, and that they affect the introduction of water much as acetic acid does.

Glycollic acid, although excluded during about 48 hours, subsequently diffuses slowly into the seed. Lactic acid did not enter most of the grains until after the lapse of 72 to 96 hours. The amount of water absorbed by the seed is diminished more by these acids than by acetic acid, thus:—

Table VI.

Solute.	Percentage of water absorbed during—					
	2 days.	4 days.	7 days.	9 days.	10 days.	11 days.
Glycollic acid	33·5	43·9	51·5	57·6	59·5	63·4
NaCl	28·2	31·9	34·1	35·2	35·7	36·0
Water	40·1	53·6	63·8	68·6	69·0	70·3
					11 days.	13 days.
Lactic acid	37·4	45·1	52·8	55·1	58·1	61·4
NaCl	30·9	34·4	35·8	36·8	36·5	

Trichloracetic Acid.—This acid was chosen on account of its similarity in configuration to acetic acid, from which it is distinguished, however, by being a strong electrolyte, acetic and the other acids and the salts which diffuse through the seed-coverings being all weak electrolytes.

On immersing seeds in a solution containing 5 per cent. of the acid, it was found to enter them very rapidly, so much so that after 48 hours they were saturated with it. This result was clearly not due to any destructive action of the acid on the seed-coverings, as when seeds saturated with a solution of trichloracetic acid were immersed in a solution of sodium bicarbonate, the acid within the seeds remained unaffected even after the lapse of 10 days. In a control experiment, seeds impregnated with acid, of which the coverings were intentionally damaged, were placed in a solution of the bicarbonate; this soon entered the seed and in a few hours neutralised the acid. Trichloracetic acid is the only strong electrolyte which has been found to possess the property of diffusing into the seed system.

Ammonia.—The membrane is more or less injured by exposure of the seeds in solutions of ammonia of weight normal strength, as acid penetrates into the corns after they have been steeped in such a solution. On the other hand, when corns which had been steeped in one-half or one-quarter normal solutions of ammonia were dried and then exposed in a normal solution of sulphuric acid during 48 hours, no acid was found to enter. The velocity with which water is absorbed from solutions of ammonia is remarkable, as shown by the results recorded in Table VII.

The ammonia passes into the corns with the water; on the other hand, when the corns impregnated with ammonia are placed in a normal solution of sulphuric acid, after 24 hours they are no longer alkaline internally, the ammonia having passed out in the reverse direction.

Table VII.

	Percentage of water absorbed.			
	2 days.	4 days.	6 days.	8 days.
Ammonia—				
Normal solution	53·5	70·1	74·8	77·2
½ " "	53·9	68·5	73·5	74·2
¼ " "	51·5	65·9	72·3	72·2
Sodium chloride (control)—				
Normal solution	29·4	32·2	33·3	34·6
½ " "	32·2	37·1	39·4	40·4
¼ " "	35·1	41·7	45·7	48·4
Water	43·1	55·6	64·1	68·3

Non-electrolytes.—As previously pointed out, cane-sugar and dextrose resemble the electrolyte sodium chloride in their power of diminishing the extent to which water is absorbed by the seed system, and in being unable to penetrate the seed-covering.

Experiments made with a number of non-electrolytes of much lower molecular weight than the sugars show the behaviour of these to be comparable with that of weak electrolytes.

The following table contains the results of experiments with volume normal solutions of ethyl alcohol, aldehyde, acetone, and ethylic acetate. For purposes of comparison, the results obtained at the same time with water and with volume normal solutions of acetic acid (representing a freely diffusible solute) and of sodium acetate (representing a non-diffusible solute) are given :—

Table VIII.

Solute.	Percentage of water absorbed.
Ethyl alcohol	74·0
Aldehyde	70·6
Acetone	72·7
Ethylic acetate	73·1
Acetic acid (control)	73·8
Water (control)	78·2
Sodium acetate (control)	39·8

The results indicate that water is absorbed by the seed-system from solutions of alcohol, aldehyde, acetone, and ethylic acetate approximately as it is absorbed from that of acetic acid, or when in contact with water alone.

Very similar results were obtained on using weight normal solutions.

Experiments in which seeds were placed in contact with alcohol, aldehyde, acetone, and ethylic acetate in the anhydrous condition have shown that these

Table IX.

Solute.	Percentage of water absorbed during—				
	2 days.	4 days.	7 days.	9 days.	11 days.
Ethyl alcohol	43 ·4	54 ·9	66 ·9	68 ·7	69 ·6
Ethylic acetate	63 ·9	70 ·7	72 ·8	72 ·1	71 ·8
Acetic acid	53 ·3	67 ·6	68 ·3	68 ·5	68 ·0
Water (control)	45 ·0	55 ·6	65 ·5	68 ·9	70 ·5
NaCl (control)	30 ·9	34 ·4	35 ·8	36 ·8	36 ·5

substances do not diffuse through the seed-coverings in the absence of water, although they all diffuse readily into the interior of the grain from their aqueous solutions.

It is also interesting to note the manner in which the velocity with which the different solutions are absorbed varies. The solution of ethyl alcohol enters comparatively slowly, at about the same rate as pure water; that of acetic acid enters more rapidly; whilst the rate of entry of the solution of ethylic acetate is markedly the most rapid of the three. Nevertheless, despite the differences of velocity, equilibrium is established between the seeds and the three solutions at approximately the same point.

A further series of observations with solutions of non-electrolytes are recorded in the following table:—

Table X.

Solute.	Percentage of water absorbed.
Glycerol	41 ·5
Glycine	41 ·8
Urea	45 ·5
Ethylene glycol	52 ·7
Sodium chloride (control)	36 ·5
Water (control)	20 ·5

The results obtained with glycerol and glycine resemble those afforded by cane-sugar and dextrose (see Table III), but differ very markedly from those obtained with such compounds as alcohol and acetic acid. The behaviour of glycine or amino-acetic acid is particularly interesting, as this compound differs to so slight an extent in constitution from acetic acid. Urea and ethylene glycol have less influence than either glycerol or glycine; glycol, however, although it differs to so slight an extent from alcohol in constitution, is far more effective in preventing the entry of water.

Summary of Conclusions.—The investigation of the selective properties of the semi-permeable seed-coverings of *H. vulgare* described in this paper should be regarded as pioneer work only; much further study is required in order to explain the varying actions of the seeds in the presence of different solutes in aqueous solution.

At present, the general trend of the evidence tends to show that solutions of the solutes which diffuse readily through the seed-coverings differ in some essential manner from solutions of non-diffusible solutes, although the nature of the difference remains unexplained. The results of some of the earlier experiments described above appear to support the view that the property of diffusion is intimately associated with a low degree of "ionisation" of the solute; yet the conspicuous instance which has been noticed of the ready diffusibility of trichloracetic acid, a highly "ionised" acid, tends to show that such correlation, if it exist at all, is not an intimate one. Further, the view does not appear to be favoured by those experiments which have demonstrated that certain non-electrolytes, such as ethyl alcohol, are readily diffusible, whilst others, such as glycerol, are non-diffusible.

In connection with the same question, it seemed possible that differences in the surface tension of solutions of diffusible and non-diffusible solutes might perhaps be associated in some way with the different behaviour of the two classes of solutions towards the seed-coverings; but it appears from a study of the surface tensions of the two classes of solutions that there is no such intimate connection between them. Neither can any indication be found that viscosity is associated with the manner in which diffusible and non-diffusible solutes behave differently towards the seed-coverings.

The only explanation of the observed difference in activity of the two classes of solutions which at present suggests itself as a working hypothesis is, that some unrecognised peculiarity in the manner in which the molecules of the two classes of solutes are combined with the molecules of the solvent water may constitute the factor which orders their different behaviour with respect to the seed-coverings. This hypothesis appears to be supported by the experiments which demonstrate that, whereas readily diffusible solutes enter the seed together with a large amount of water, seeds placed in solutions of non-diffusible solutes absorb water with some difficulty. Moreover, the observation that an aqueous solution of alcohol diffuses readily through the seed-coverings which are impervious to this solute in the anhydrous state, appears to show that some form of combination of solute and water is necessary to condition diffusion of the solute through the seed-coverings.

The Origin of Osmotic Effects. II.—*Differential Septa.*

By Henry E. Armstrong, F.R.S.

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I have had the privilege of following the progress of the inquiry of which an account is given by Prof. A. J. Brown in the previous communication and of watching the development of the exquisite and invaluable method of studying the osmotic process which he has devised; I trust that I shall not be presuming if I discuss the results which he has arrived at and attempt to interpret them in the light of views already placed before the Society in my communication on "The Origin of Osmotic Effects"* and in the series of "Studies of the Processes Operative in Solutions."†

Prof. Brown's observations appear to be extraordinarily significant, as affording the means of dividing substances broadly into two classes according as they will or will not diffuse through a membrane such as that which forms the outer covering of the seed of barley (*Hordeum vulgare*) and with the aid of the classification thus secured of arriving at an explanation of the selective process.

Inasmuch as the barley grain contains but a small amount of soluble crystalloids, the absorption of water by the grain may be regarded as mainly conditioned by the extremely minute granules of starch enclosed within it; presumably these have great attraction for certain molecules in the liquid and become coated superficially therewith. From this point of view the method developed by Prof. Brown involves the study of a struggle *for hydrone* between a mass of fine particles of solid and the solution of a substance present in the liquid state in solution in water; the observations are the first of their kind, I believe.

It is clear, although the method affords only approximate results, that the conclusions to be deduced as to the relative "concentrating" efficiencies of the several solutes are in general accordance with those arrived at in other ways. The observations made in my laboratory show that chlorides are more active than nitrates in solution and that sodium salts are more active than either potassium or ammonium salts—more active, that is to say, in the sense that they exercise a greater concentrating effect; this is precisely the result arrived at by Prof. Brown.

No division of the substances into electrolytes and non-electrolytes can be made in any way corresponding to the extent to which water is absorbed from the solutions by the grains—cane-sugar is nearly as active as common salt. In like manner, not only are strong acids and most salts indiffusible through the membrane covering the grain but also not a few non-electrolytes; the membrane is slowly permeated by certain weakly oxygenated organic acids, by salts such as mercuric chloride and cadmium iodide, by iodine, by ammonia and by a number of non-electrolytes of low molecular weight.

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* 'Roy. Soc. Proc.,' 1906, A, vol. 78, p. 264.

† *Ibid.*, p. 272 (I); II—V, vol. 79, 1907, pp. 564—597; VI—X, vol. 81, 1908, pp. 80—140.

The compounds which penetrate the membrane, whether electrolytes or non-electrolytes, are all substances which attract water presumably only to a minor extent and which exist to some extent in solution in an unhydrated condition; those which cannot penetrate it, on the other hand, probably all form hydrates of considerable stability in solution.

I picture surfaces generally, colloid surfaces in particular, as not merely wetted by water but as more or less hydronated and hydrolated—using these terms in the specific sense explained in No. VIII of my "Studies on Solutions"; that is to say, they are not merely wetted by water complexes* but associated with *hydrone*, the simple fundamental molecule of which water is composed. The intramolecular passages in a colloid membrane, if thus hydrolated, would be guarded by the attached hydrone molecules; molecules in a solution bathing the membrane which attempted to effect an entry through such passages, if hydrolated, would be seized upon and held back in virtue of the attraction which the two hydrolated surfaces—that of the membrane and that of the solute—would exercise upon one another. The hydrolated passages, however, would be indifferent to molecules which were not hydrolated—consequently, a substance such as acetic acid, of which probably only a small proportion is present in solution in the hydrolated state, would gradually pass through them.

The apparently exceptional behaviour of trichloracetic acid, which must be more fully if not more firmly hydrolated than acetic acid, is very striking and may be taken as proof that the hydrolation must extend over a certain area to secure protection against penetration; it should be noted, however, that the result is in accordance with the behaviour of the acid as a substitution derivative. Hydroxy- and amino-acetic acids (glycollic acid and glycine), which are far weaker acids, are nevertheless far less easily diffusible—presumably because not only the carboxyl group of the acid but also the adjacent hydroxyl- or amino-group is hydrolated. The behaviour of glycol, $C_2H_4(OH)_2$, in comparison with that of alcohol, $C_2H_5(OH)$, may be interpreted in a similar manner. The concentrating effect exercised by the sugars has already been considered from this point of view in our previous "Studies" (VIII, pp. 111, 112; X, p. 130).

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* Compare 'Chemical News,' Jan. 15 and 22, 1909, pp. 28 and 37; 'Science Progress,' Jan., 1909, No. XI, p. 484.

The exceptional rapidity, to which Prof. Brown directs attention, with which ethylic acetate acts in promoting the entry of water into the grain is also easily explicable from the same point of view. Entering together with water, it should render water within the grain more active and more attractive of external water (by promoting its dissociation, $(H_2O)_x \rightarrow xH_2O$) than the water would be which entered alone from a solution of an indiffusible solute, as in such water (on account of its homogeneity) the osmotic stress would be at a minimum.*

It is obvious that the argument now put forward may be applied to the discussion of a great number of more or less obscure physiological phenomena. It may be desirable to consider the rise of the sap in trees from such a point of view. The argument affords an explanation of the well-known efficacy, for example, of mercury salts, of iodine and of alkaloids as drugs. It should point the way to the production of medicaments adjusted to their purpose—according as it is desired that they should penetrate this or that membrane. It may lead to the discovery of a method of using stains as the means of determining whether this or that membrane or layer in a cellular tissue is to be regarded as a mere sieve or as differentially penetrable, inasmuch as stains—which hitherto have been used all but empirically—must vary greatly in penetrative power and it should be possible to grade them, according to their diffusibility, by observations similar to those made by Prof. Brown.

* [February 15, 1909.—Attention has been specially drawn in No. VIII of our Studies (p. 108) to the behaviour of methylic acetate as a weak hydrolyte in comparison with the strong hydrolyte cane-sugar; the observations now under discussion appear to afford complete confirmation of the argument there put forward that in discussing the phenomena of hydrolysis it is necessary to take into account not only the condition of the medium but also the nature both of hydrolyte and of hydrolyst, which are reciprocally concerned in the change. The argument should be extended to colloid and other surfaces. Sir James Dewar has shown that solids differ greatly in their power of attracting and holding gases at low temperatures; hydrolytes and dissolved substances generally, we must suppose, also differ in the extent to which they undergo "hydration"; wetted surfaces generally must also differ in the extent to which they become hydrolated; consequently, it is to be supposed that more or less considerable variations will be met with when differential septa are studied comparatively. Apparently the barley septum is not penetrated even by ammonium chloride, so that it is more exclusive than that of red blood cells, which are rapidly penetrated by this salt but scarcely if at all by ammonium sulphate. The difference between ammonium chloride and ammonia is very striking, the latter resembling ethylic acetate in passing rapidly into the seed and in promoting the ingress of water; this behaviour is easily understood, as it exists in solution partly in the free state and partly, it may be supposed, as the hydrone $H_3N : OH_2$, the hydroxide being present in only very small proportion. If ammonia were contained in solution as the hydroxide, its behaviour would undoubtedly be that of caustic soda.]

On the Determination of a Coefficient by which the Rate of Diffusion of Stain and other Substances into Living Cells can be Measured, and by which Bacteria and other Cells may be Differentiated.

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(Communicated by Major Ronald Ross, C.B., F.R.S. Received December 9, 1908,—Read February 11, 1909.)

[Plate 3.]

In former papers (3, 4, 5) it has been shown that when blood is spread upon a film of agar jelly which contains Unna's stain and certain salts, the cells will absorb the stain, and that the absorption increases with the temperature and the time during which the cells have been resting on the film. The following facts have also been published:—(1) That *alkalies*, like *heat* and *time*, increase the diffusion of stain into the cells; (2) that *acids* and *neutral salts* delay the diffusion; and (3) that the staining of the nuclei of leucocytes is a sign of death. Soon after death the staining ceases, and the cells rupture or lose their stain.

Evidence has also been given that these phenomena are due to the diffusion of stain into the jelly-like cytoplasm being hastened or delayed, as the case may be, by the agency of these factors, and that death, coincident with the staining of the nucleus, is followed by liquefaction of the cytoplasm and other changes which cause the cells to lose their stain and enter a phase which has been called the condition of achromasia (6).

I have made further investigations in this subject, and have ascertained that if the constituents of the agar film are arranged in a constant manner and the other factors are constant, the staining of the cells will be constant provided that the latter are in the same healthy condition when placed on the agar. It has also been found that when one class of blood cell stains on a given agar film, others do not. By altering one or more of those factors which hasten or delay diffusion of stain into the cytoplasm, that class of cells which previously refused the stain will now absorb it. Therefore the rate of diffusion of stain into the cells differs with the class of cell. Cells other than blood cells, especially bacteria, have also been tried and have been found to be subject to the same conditions; and it has been possible, by altering the arrangement of the factors, to differentiate cells by their rate or coefficient of diffusion. The object of this paper is to give methods by which the coefficient of diffusion of living cells can be obtained, and the differentiation accomplished.

Scheme of the Method employed.—The cells to be tested are placed on a film of jelly on a slide. The film is prepared from a test-tube which contains 10 c.c. of jelly. The 10 c.c. of jelly contains, besides stain, some of the factors such as *alkalies*, *acids*, and *salts*, which hasten or delay the diffusion of stain into the cells. In order to simplify matters, I measure these factors in units and I endeavour to arrange them in such a way that 1 unit of any one factor is equal in value, as regards hastening or delaying diffusion, to 1 unit of any of the other factors. The factor *heat*, which hastens diffusion, is applied after the cells have been spread upon the film by keeping the slide at various temperatures for various periods of time. This factor, as well as that of *time*, is also arranged in units, so that 1 unit of either of them is equal in value to 1 unit of the other factors contained in the agar. Hence if 1 unit of a given substance delays diffusion of stain into a cell, the delay it causes can be exactly neutralised by the addition, either to the agar or to the slide, of 1 unit of a factor which hastens diffusion.

Consequently an equation is formulated by means of which the total number of units which, with a certain amount of stain, will cause given, or stipulated, staining of the cells, is equivalent to, or said to be equivalent to, their coefficient of diffusion. Therefore to find the coefficient of a cell, it is necessary to prepare films from a succession of tubes of 10 c.c. of jelly, each tube having a certain, known, number of units added to it, and to examine each film until the stipulated staining is obtained. Since the units are equal in value, it matters little, within reasonable limits, which factor is added, provided that the total number of units is known. Then, after subtraction of those units which delay diffusion, the remainder added to the quantity of stain is the coefficient of diffusion of the cell experimented with.

Conversely, if the coefficient of diffusion of a cell is known, one is enabled, by means of the equation, to know how many units of one or several of the factors it is necessary to add to the jelly, or apply to the slide, to obtain stipulated staining of that variety of cell in a given time. I have mentioned that the staining of healthy cells, only, appears to be constant. When the rate of staining of cells of persons suffering from disease has been found experimentally, the equation indicates in a moment the difference between the coefficients of healthy and diseased cells, and this difference can be expressed in grammes, degrees, or minutes, etc., according to the nature of the factor into which the coefficient of diffusion may be ultimately resolved.

Definitions.—When a film of agar jelly contains stain and other substances, its Index of Diffusion (*fx*) may be defined as the sum of its constituents which delay diffusion subtracted from the sum of constituents which accelerate diffusion added to the quantity of stain contained in the jelly.

The Coefficient of Diffusion (*cf*) of a cell is that index of diffusion *plus* the time and temperature required to cause staining of the nucleus, or staining of the cytoplasm in unnucleated cells (*e.g.*, red corpuscles), when the specimen is prepared by a standard method.

Standard Method of Preparation.—This consists in: (1) Mixing the cells with a neutral solution containing 3-per-cent. sodium citrate and 1-per-cent. sodium chloride.* If blood is experimented with, it is mixed with an equal volume of the solution. In the case of bacteria and other cells, the mixture is made as convenient.† (2) The mixture is then placed on a cover-glass, which is inverted and allowed to drop flat on a film of agar jelly containing Unna's stain and salts, and which, after boiling, has been allowed to set on a slide. Since the surface of the film is convex, the solution spreads to the periphery of the cover-glass, leaving the cells gently pressed out between the glass and jelly, and this affords an excellent means of examination by the microscope.‡ Only the cells in the centre of the preparation should be examined.

It is stipulated that the jelly contains stain, but the amount of stain added to the agar may be variable. The chemical nature of methylene blue may or may not affect diffusion. The point is difficult to determine accurately, but in this procedure it is of little importance, because the stain employed is always the same, namely, Unna's polychrome methylene blue (Grubler). On the other hand, it is obvious that the more concentrated it is—that is, the more stain there is in the 10 c.c. of jelly, the more rapidly, *ceteris paribus*, will the cells stain. It is also obvious that the effect of an increase of the concentration of the stain can be neutralised by the addition of one or several factors which delay diffusion. Consequently I also measure the stain in units, so that an increase of its concentration by 1 unit can be neutralised by the addition of 1 unit of a factor which decreases diffusion.§

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* Merck's reagents have been used throughout these researches.

† The mixture of the cells with this solution is merely used as a vehicle to keep them alive. As the solution spreads to the periphery of the cover-glass, it does not materially influence the diffusion of stain from the agar, a point which has been tested experimentally by rendering it alkaline. The cells, however, should not be kept in it longer than necessary.

‡ The suggestion of mixing stain with the jelly was made to me, as already noted elsewhere, by my brother, Prof. Ronald Ross.

§ Unna's polychrome methylene blue is only supplied in solution, which is standardised. It cannot be made in a powder.

Convenient Method of Preparing the 10 *c.c. of Jelly.*—I have found it more accurate and simple if the factors which hasten or delay diffusion are added to the jelly from standard solutions, and I make the tubes of 10 c.c. of jelly as follows:—50 c.c. of a 2-per-cent. solution of powdered agar in water, filtered and sterilised, is prepared. This solution has such a consistency that another 50 c.c. of water could be added to it without preventing it from setting on a slide when cold.

Since I have shown that blood cells will not live on agar jelly unless it contains a combination of sodium citrate and sodium chloride (3, 7), I add to the 50 c.c. of jelly 1 gramme of sodium citrate and 0·8 gramme of sodium chloride, and accurately neutralise to litmus with citric acid. The whole is then rendered acid by the addition of 0·083 gramme of citric acid. The reason for this will be given in the next paragraph but one.

The molten jelly is then decanted into test-tubes, each of which contains 5 c.c., so that it is possible to add the stain and certain quantities of the standard solutions which contain the factors to these tubes, and provided the total quantities of the several solutions added do not cause a tube to contain more than 10 c.c. of jelly, its ultimate setting on a slide is assured. The standard solutions are so arranged that their total quantity required in an experiment never does exceed 5 c.c. On the other hand, if the amount is less than 5 c.c., the balance up to the maximum in the tube of 10 c.c. is made up with water. In other words, a test-tube originally contains 5 c.c. of jelly which is acid and holds a certain quantity of salts in solution. The stain and quantities of the standard solutions, which correspond to the number of units of factors which it is intended to try, are added. The total content of the tube is then made up with water to 10 c.c. and boiled. So that a test-tube never contains more nor less than 10 c.c. of jelly when a film is prepared from it, though it may contain a variety of units of stain and standard solutions.

I have stated that the agar is rendered acid at the outset; this is done to reduce the number of factors. Acids and alkalies delay and accelerate diffusion respectively. Since they neutralise each other, the neutral point would also have to be taken into consideration. As this would complicate the equation, I render the agar acid at the outset, so acid that I cannot get any cell to stain on it with 1 unit of stain, and deal only with alkali. The neutral point I ignore, although by knowing the initial acidity of the agar, and that the units of all the factors are equal, the point can be readily determined by referring to the equation. I therefore deal with one factor, alkali, instead of two and a neutral point.

To recapitulate shortly: 50 c.c. of a 2-per-cent. solution of agar is prepared which contains 1 gramme of sodium citrate and 0·8 gramme of sodium chloride. It is neutralised and rendered acid with 0·083 gramme of citric acid. It is then collected in quantities of 5 c.c. In order to determine the *cf* of a cell, a tube of 5 c.c. is melted and certain quantities of stain and standard solution of alkali added. The content of the tube is then completed with water up to 10 c.c. Consequently the tube contains 1 per cent. sodium citrate and 0·8 per cent. sodium chloride in addition to the acid, stain, and alkali, and this content of salts allows leucocytes to live on the jelly. The whole is then boiled until it froths up the tube and a film prepared from it by pouring a drop on a slide and allowing it to set. The cells are placed on to the film and the slide is kept at a convenient temperature for a period of time. If the nuclei or cytoplasm are not yet stained, a higher temperature may be tried combined with a longer period of time, or a fresh tube prepared with more units of alkali added, and so on until staining is obtained. Should the contents of a tube cause the cells to stain very deeply, or if they soon become achromatic, a fresh tube is made containing less stain, or more salts, or less alkali, or acid may even be employed, and so on. But provided the arrangement of the contents of the tube which just causes staining of the nuclei is known, and if the time and temperature are also known, the equation will give the *cf* required.

Units.—In preparing these units I have mainly considered their practical application in the endeavour to curtail the procedure as much as possible. In the instance of alkali and salts, I give the actual amount in grammes which 10 c.c. of jelly should contain as 1 unit. I also give a convenient standard solution and the amount of it in cubic centimetres to be contained in the 10 c.c. of jelly to constitute 1 unit.

Alkali, Sodium Bicarbonate, hastens diffusion.—Unit, 0·005 gramme. Standard solution 5 per cent., unity being 0·1 c.c. It is convenient to remember that this solution is neutralised by a 4·175-per-cent. solution of citric acid, and that 1 unit of alkali is neutralised by 0·1 c.c. of such a solution. Since the agar at the outset is acid to the extent of 0·083 gramme to 50 c.c., a tube of 10 c.c., made up as described, must contain 0·0083 gramme of acid. This is exactly neutralised by 0·2 c.c. of the standard alkali solution; that is, the agar at the outset, before any stain or other factor is added, delays diffusion to the extent of 2 units. Or, the addition of 2 units of sodium bicarbonate will render the agar neutral.

Sodium Citrate, delays diffusion.—Unit 0·03 gramme. Standard solution 10 per cent., 0·3 c.c. being unity. Since 50 c.c. of agar contains 1 gramme at the outset, the 10 c.c. of jelly may be said to contain about 3 units.

Sodium Chloride, delays diffusion. Unit 0·08 gramme. Standard solution

10 per cent., unity being 0·8 c.c. The 10 c.c. of jelly contains this from the outset.

Heat, hastens diffusion.—Each unit 5° C. 10° C. is unity, 15° C. is 2 units, 20° C. 3 units, etc. For practical purposes I call 37° C. 7 units.

Time, increases diffusion.—10 minutes being 1 unit, 20 minutes 2 units, and so on.

Stain, Unna's polychrome methylene blue (Grubler), behaves as if it increased diffusion.*—Unit 0·1 c.c.

Equations for ascertaining the Coefficient of Diffusion.—The nuclei of polymorphonuclear leucocytes recently shed from a healthy person, just stained in 10 minutes when resting on a film of agar, 10 c.c. of which contained 0·2 c.c. of stain, 1 per cent. sodium citrate, 0·8 per cent. sodium chloride, and 6 units of sodium bicarbonate. The slide was kept at a temperature of 37° C. What was their coefficient of diffusion?

Then $cf =$ that $fx+h+t$ which just causes staining of the nuclei,

but $fx = (s+a)-(c+n)$;

$$\therefore\ cf = (2s+6a)+(7h+t)-(3c+n) = 16-4 = 12.$$

Where s is the unit of stain, a the unit of alkali, h the unit of heat, t the unit of time, c the unit of sodium citrate, and n the unit of sodium chloride.

The 10 c.c. of agar in this case was made up as follows:—5 c.c. from the original 50 c.c. of agar which contained sodium citrate 1 gramme, sodium chloride 0·8 gramme, and citric acid 0·083 gramme. The jelly was melted and the following quantities of standard solutions added: 0·2 c.c. stain, 0·6 c.c. 5-per-cent. solution of sodium bicarbonate, and 4·2 c.c. water. Total 10 c.c.

The eosinophiles, however, did not stain under quite the same conditions for it was found in the foregoing experiment that they were either achromatic or ruptured. A fresh tube was made with 1 unit less alkali, when it was found that the eosinophiles would just stain in 10 minutes. What was their cf?

$$cf = (2s+5a+7h+t)-(3c+n) = 11.$$

The lymphocytes, large and small, required 0·2 c.c. of alkali more than the polymorphonuclear cells, the other factors being as before, what was their cf?

$$cf = (2s+8a+7h+t)-(3c+n) = 14.$$

The foregoing tubes contained a very low content of stain, the chromatin network stained better if its concentration was increased. In order to obtain staining of the nuclei of lymphocytes in 10 minutes on agar from a tube which contained 4 units, instead of 2 units, of stain, the amount of alkali added was of course less in proportion to the increased concentration. With 4 units of stain, the other factors, except alkali, being as before, the equation now stood as :—

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* As already pointed out, stain should not be a unit of diffusion, for it is doubtful whether it affects diffusion. It contains salts, and is alkaline when made, and alkalies and salts are antagonistic. However, for reasons already given, it may be included in the category.

$$cf = (4s+6a+7h+t)-(3c+n) = 14.$$

The red corpuscles appear to have a very high *cf.* I caused them to stain on agar which contained 1 c.c. of stain (10 units) and 11 units of alkali, in the presence of 1-per-cent. sodium citrate and 0·8-per-cent. sodium chloride at 37° C. in 10 minutes. This was the equation :—

$$cf = (10s+11a+7h+t)-(3c+n) = 25.*$$

Examples.—A growth of staphylococci had a *cf* of 16.

How much alkali must 10 c.c. of jelly contain to cause the germs to stain in 10 minutes if the jelly already contains 5 per cent. of stain, 1·5 per cent. sodium citrate, and 0·8 per cent. sodium chloride, when the slide is incubated at 37° C.?

$$a = (16cf+4{\cdot}5c+n)-(5s+7h+t),$$
$$a = 8{\cdot}5 \text{ units, or } 0{\cdot}0425 \text{ gramme of sodium bicarbonate.}$$

A strain of typhoid bacilli had a *cf* of 21. A tube of agar contained 6 units of alkali solution and the usual quantities of sodium citrate and chloride. How much stain should be added to the tube to produce staining of the bacilli in 20 minutes at 37° C.?

$$s = (21cf+3c+n)-(6a+7h+2t),$$
$$s = 10 \text{ units of stain, } i.e., \text{ 1 c.c.}$$

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* I do not think this is strictly accurate, for it depends on the coloration of the stroma. Nucleated red cells have a comparatively low *cf*, resembling that of the polymorphonuclear cells, though I am also doubtful of this point, because I have only been able to obtain these nucleated cells from persons suffering from disease, and, as I have already shown (8), all the blood cells in chronic illnesses, especially phthisis, malaria, and Hodgkin's disease, have so far shown a general fall in their coefficient of diffusion. Again, attention may be drawn to the comparatively low *cf* of the granular erythrocytes which constitute about 1 per cent. of all the red cells in healthy blood as demonstrated by this method of examination (1). The rate of staining of the granules is about the same as those of the polymorphonuclear leucocytes, but the rate of staining of the stroma of these granular cells is much higher than that of their granules, yet lower than the rate of staining of the stroma of ordinary erythocytes. This fact is the more interesting, because it is almost in these granular red cells alone, which have a low *cf*, that "red spots" are to be seen, although I have on three occasions seen them in ordinary red cells, and the spots appear to be dependent on diffusion (4). These cells also become achromatic more readily than ordinary erythrocytes. The granules have been described (1) as the remains of a nucleus. The question, therefore, of the *cf* of the red cells I will leave for the present as indicated.

Precautions.—As regards Life and Death: In a previous paper (3) it has been shown that the staining of the nuclei of leucocytes, when examined by this method, is a sign of death, and that the nuclei of dead cells will stain, *ceteris paribus,* before those of living cells. Consequently all the experiments given in the present paper have been made with fresh normal cells, and in the case of micro-organisms with cultures not more than 48 hours old. It may also be mentioned that the liquefaction of the cytoplasm which occurs after death materially alters the conditions of staining of leucocytes, and that the *cf* of living blood cells falls gradually after the blood has been shed. The fact has already been mentioned that in chronic wasting diseases the coefficient of blood cells may be very low.*

As regards Excess of Alkali causing rapid death and liquefaction of the cytoplasm with consequent prevention of staining (achromasia): The addition of excess of alkali may cause death, staining of the nuclei liquefaction, and the loss of stain on the part of the cells (3, 6). This may occur before a preparation can be focussed, in which case the cells appear unstained and will refuse to stain, no matter how much more stain or alkali are tried. Therefore it is better to begin with a low index of diffusion and to try tube after tube, each containing a little more alkali, until staining is obtained. Further, the amount of sodium bicarbonate should not exceed 20 units, because, as has already been pointed out in a former paper (3), if added to excess, it may act as a neutral salt and delay diffusion.

As regards Deficiency of the Salts sodium citrate and sodium chloride: If the jelly contains no salts, the blood lakes and the leucocytes are killed outright. If it contains sodium chloride only, the cells are killed rapidly, and the same may be said if sodium citrate only is employed (7). In examining blood, therefore, the combination is essential.

As regards Excess or Deficiency of Heat: A temperature above 40° C. may allow the cells to diffuse through the agar (2). A temperature below 15° has not been experimented with, because, even at a temperature of 20° C., it requires a minimum of 3 units of stain to cause staining of the nuclei of leucocytes in spite of the addition of a large amount of alkali, for the alkali is not sufficient, *per se,* to cause the cells to absorb sufficient stain to colour the nuclei unless the stain is concentrated.

As regards excess of Time: A period of more than half an hour has not been employed for fear of death and liquefaction of the cytoplasm, for the cells may die and become achromatic before there has been time for sufficient stain to diffuse into them to cause staining of the nuclei, in which case, of course, the cells will never stain.

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* I have found that the life of leucocytes of persons suffering from some chronic diseases, when bathed in their own plasma, is considerably shorter than the life of healthy persons' leucocytes bathed in their own healthy plasma (8). I have reasons for believing that there is an association between this and a low *cf.*

As regards Excess of Stain: More than 10 units of stain may cause precipitation of the agar as the film cools on the slide, and the precipitate carries some of the stain down with it, vitiating the results, for it has been shown that agar is not very soluble in cold stain (3).

As regards Examination: The observation of cells floating near a bubble under the cover-glass should be avoided. The fact that blood cells in such a situation will stain before others has already been noted (3). I consider this to be due to these cells floating in a small quantity of alkaline citrated plasma collected round the bubble.

Consequently the experiments have all been made within the compass of the above restrictions. So far no cells, whether blood, bacteria, or other cells, have been met with which would not give a coefficient of diffusion by this method. It may also be advised that when unnucleated cells contain granules in their cytoplasm, the staining of the granules gives a more constant rate than the staining of the cytoplasm. By this means it is seen that the *cf* of the blood-plates is identical with that of the polymorphonuclear cells.

The Construction of other Units.—It may be necessary to add other substances to the jelly to test their effect on cells. For instance, it may be useful to try other salts, in which case their rate of antagonism to diffusion must be found and a unit made. This may be done by comparing their action with that of a unit of one of the other factors, after which the new unit may be added to the equation. In the case of sulphate of atropine, it was found that a tube of 10 c.c. of agar, which had a correct *fx* to cause staining of lymphocytes in 10 minutes, but which also contained 0·013 gramme of sulphate of atropine, required the addition of 1 more unit of alkali to cause the nuclei to stain in 10 minutes. Consequently the unit of atropine sulphate may be said to be 0·013 gramme.

The Determination of the Coefficient of Diffusion of Leucocytes involves Death.—Since the staining of the nucleus is the moment by which the *cf* of leucocytes is obtained, and since the staining of the nucleus is a sign of death (3), the cells are necessarily dead at the expiry of the time involved in finding their coefficient.

The Reconciliation of their Coefficient of Diffusion to Cells which may be Alive at the Termination of the Time required.—It has been shown that leucocytes will live for a considerable period and show amœboid movement with their granules stained (3, 5). If 1 digit is subtracted from their *cf* and the jelly arranged according to such an equation, the granules but not the nuclei will stain in the given time. By this means death is not necessarily involved.

Example to show that the Diffusion of Substances other than Stain may be Dependent on the Coefficient of Diffusion of Cells.—Given eosinophiles have a *cf* of 11. They are resting on agar which contains the usual quantities of sodium citrate and chloride, viz., 3 and 1 units respectively, but it also contains 0·02 gramme of sodium bicarbonate (4 units), 0·6 c.c. of stain, and 0·007 gramme of atropine sulphate. The fact that a mixture of atropine and methylene blue will excite a remarkable exaggeration of amœboid movement in leucocytes has already been published (5). How long will it take to produce marked exaggerated movements in the given eosinophiles at a temperature of 20° C.?

Then, since it is necessary for the given cells to be alive at the expiry of the time required, 1 digit must be subtracted from their *cf*—

$$t = (10cf + 3c + n + 0{\cdot}5z) - (6s + 4a + 3h),$$
$$t = 1{\cdot}5 \text{ units of time or 15 minutes.}$$

Where z is the unit of atropine sulphate, 0·013 gramme. A 1-per-cent. solution was found convenient as a standard, and 0·7 c.c. was used.

The Coefficient of Diffusion may be resolved into the Value of any one of the Units.—Since by the foregoing equations any one of the units can be resolved into the value of any one of the other units which go to make the fx, and since $fx + h + t = cf$, therefore any cf can be expressed in the value of any of the units; into alkali for instance. But the unit of alkali is 5 milligrammes of sodium bicarbonate. Consequently the coefficient of diffusion of the bacteria contained in the growth of *Bacillus typhosus*, used in one of the examples, may be said to be equivalent to the alkalinity of 105 milligrammes of sodium bicarbonate.

Summary and Suggestions.

The difficulty has been in the construction of the units. So far I have found them to be sufficiently accurate for practical purposes within the compass of these experiments, of which a very large number have been made, extending over a period of several years, involving the use of many varieties of cells.

The determination of the coefficient of diffusion is brought about by allowing living cells to rest on a jelly which contains stain. Several factors, some of which may be contained in the jelly, hasten or delay the diffusion of the stain into the cells, and the coefficient of diffusion is the sum of the factors which causes the stipulated staining of the cells added to the amount of stain employed. The factors which hasten diffusion are *heat*, *alkalies*, and *time*; and those which delay it are *acids* and *neutral salts*. But the rate of diffusion depends also on the concentration of the stain, for if this is weak a large sum of factors to produce staining is required; but if it is very concentrated the cells may stain even in the presence of acid. Since all these items are variables, I have constructed a formula by which, if some of them are known quantities, the others can be readily determined.

By this means the coefficient of diffusion of a cell can be obtained, and it varies with the class of cell.

I have also stated that the diffusion of substances other than stain may appear similarly to depend not only on their concentration but on the coefficient of diffusion of a cell. But other substances may be alkalies, acids, or salts, and may affect the diffusion of neighbouring substances and be so affected themselves. I have given a means by which this effect can be determined and a unit made. Then, provided the unit of a given substance is known, and provided the coefficient of diffusion of a given cell is known, the comparative rate of effect of the given substance on the given cell can be ascertained by referring to the equation.*

In medicine, for instance, drugs and sera are frequently given with a view to affecting certain cells, yet, as far as I know, no steps are taken either to ascertain the rate of effect of the drug on the cells, or suitably to modify the alkalinity of the blood by treatment, in order to produce maximum results according to the temperature of the patient. The point appears worthy of consideration.

In the case of bacteria, it is frequently heard that certain bacteria are more resistant to antiseptics and drugs than others. It is possible that this varied resistance may be summed up in the expression "coefficient of diffusion." If antiseptics could be rendered alkaline according to the coefficient of the bacteria which they are intended to kill, and according to the temperature, it might lead to a reduction of the concentration of the antiseptic, with consequent saving of cost and increase of efficiency.

Since the blood fluids affect bacteria, it seems desirable to know the coefficient of diffusion of the bacteria when estimating the effects of the fluids on the cells. Again, since erythrocytes will diffuse bodily into agar jelly and remain suspended in it (2), and since droplets of liquid will diffuse into the colloid cytoplasm of leucocytes and remain suspended in it (red spots, 4), it seems possible that germs which proffer the same jelly-like properties as red cells may enter the phagocytes by the process of diffusion and be subject to the same factors which influence it, for the temperature and alkalinity of the blood vary in health and disease. Therefore, it may be important to know the rate of diffusion of leucocytes when estimating phagocytosis.

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* The knowledge that heat accelerates the diffusion of substances into cells has already been applied in some researches by Dr. C. J. Macalister and myself in order to demonstrate an excitant for leucocytes in the plasma of cancer patients ('Proc. Roy. Soc Med.,' December, 1908).

I have noted that there appears to be a relationship, in leucocytes at least, between the coefficient of a cell and the length of its life as measured by a procedure which I have already published (5, 8). It has also been shown in a former paper (4) that if stain is passed over a jelly such as agar, the rate of the coloration of the jelly depends on its consistency, that is, as to whether it is solid or diffluent. In the same paper it was stated that the effect of stain on cytoplasm also depended in a like manner on its consistency. Since there may be a relationship between the coefficient and vitality, the consistency of a cell may depend to some extent upon its vitality. Therefore, the determination of the coefficient of diffusion may prove important in the prognosis of tumours if the cells can be suitably kept alive, since it may give an indication of the consistency of the cytoplasm, and a lowered coefficient, as occurs in the blood cells in anæmia, may foretell a lowered vitality.

Further experimentation is also required to determine the property on which depends the varying influence of alkalies, salts, etc., in hastening or delaying diffusion.

I hope this method may ultimately prove of value, not only in bacteriology as a means of differentiating bacteria, but also in the investigation of the diffusion of substances into living cells.

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(2) H. C. Ross, "Diffusion of Red Blood Corpuscles through Agar," 'British Medical Journal,' May 5, 1906.

(3) " "On the Death of Leucocytes," 'Journal of Physiology,' vol. 37, p. 327, 1908.

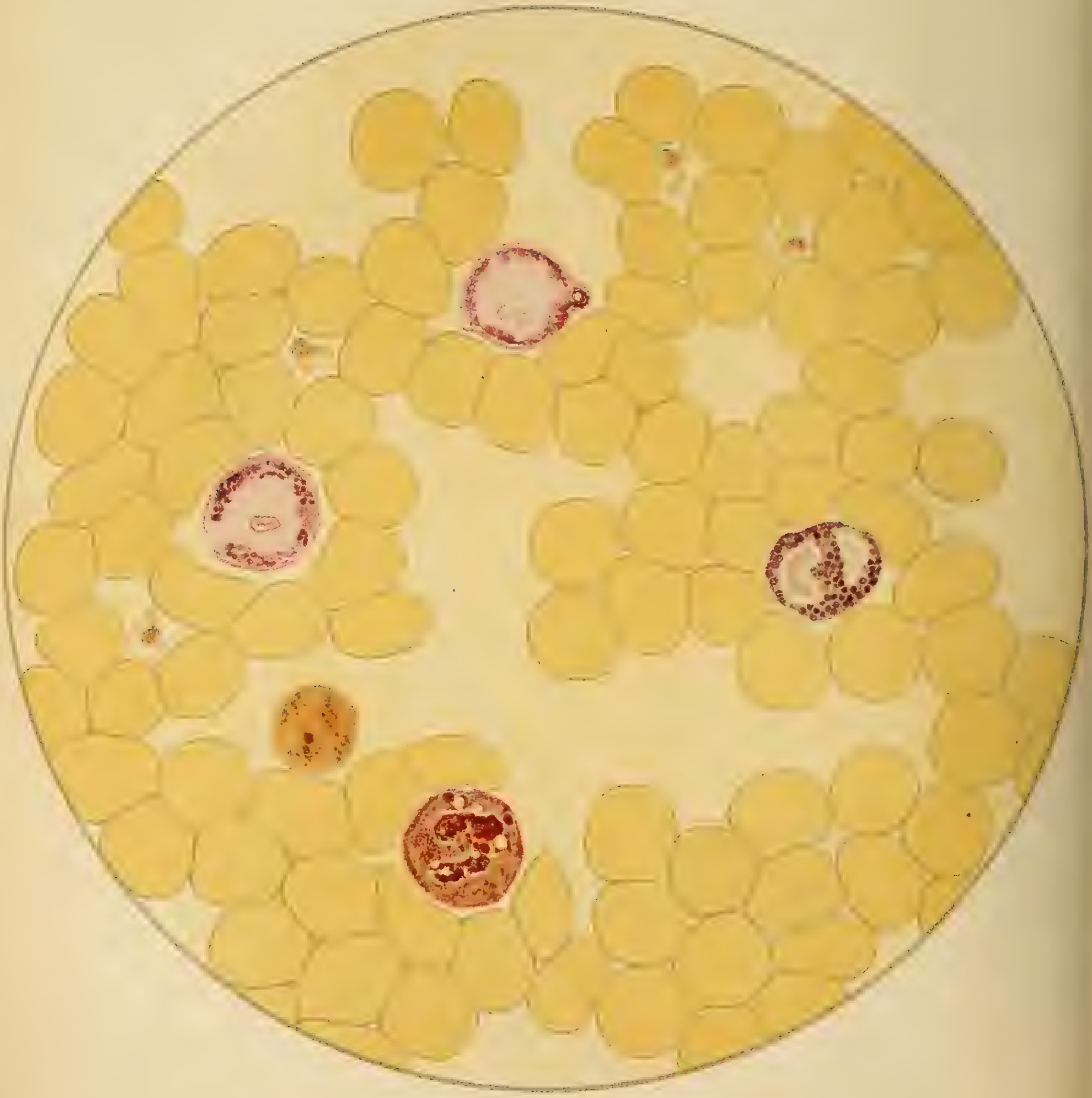
(4) " "On the Vacuolation of Leucocytes, etc.," 'Journal of Physiology,' vol. 37, p. 333, 1908.

(5) " "On a Combination of Substances which Excites Leucocytes, etc.," 'Lancet,' January 16, 1909.

(6) " "On the Cause of Achromasia in Leucocytes," 'Lancet,' January 23, 1909.

(7) " "On the Modification of an Excitant for Leucocytes, etc.," 'Lancet,' January 30, 1909.

(8) " "Some Comparative Measurements of the Lives of Leucocytes, etc.," 'Lancet,' February 6, 1909.



DESCRIPTION OF PLATE 3.

Coefficient of diffusion. Drawn by P. Nairn from a preparation of fresh blood cells which have been resting for 10 minutes at 37° C. on an agar film with an index of diffusion of 4. The nucleus of one polymorphonuclear leucocyte has just stained and the cell is showing three small red spots. The nuclei of two large lymphocytes have not yet stained, one cell is showing 1 centrosome and the other 3 centrosomes. The film also demonstrates an eosinophile leucocyte which is becoming achromatic, *i.e.*, its nucleus has lost its stain; and one granular red cell which contains two red spots. 2 mm. apochromatic objective, No. 4 eye-piece, 250 mm. draw-tube, 1 amp. Nernst lamp.

The Origin and Destiny of Cholesterol in the Animal Organism. Part III.—*The Absorption of Cholesterol from the Food and its Appearance in the Blood.*

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In his 'Text-Book of Physiology' Schäfer has suggested that the constant presence of lecithin and cholesterol in the bile may well be associated with the destruction of the red blood corpuscles which contain relatively large amounts of these substances, the latter, according to Hepner,* being present in the free state and not in the form of esters. This idea has recently received strong support from the investigations of Chasoburō Kosumoto† on the influence of toluylene diamine on the output of cholesterol in the bile. This reagent was found by Schmiedeberg to produce icterus, and Stadelmann, working in Schmiedeberg's laboratory,‡ observed that at the beginning of the action of the drug an increased production of bile took place. This, however, was only temporary, and soon the normal physical properties of the bile underwent an alteration; it became sticky, darker, and more concentrated.

Afanassiew§ showed that the effect of the drug is to destroy the red blood corpuscles. The increased viscosity, which Afanassiew correlated with certain changes observed by him in the liver tissue, causes a hindrance to the flow of bile, and since the bile formation is, perhaps, increased beyond the normal, leads to icterus. When the action of the toluylene diamine ceases—according to Afanassiew owing to the removal of the hindrance which opposes the bile flow—the amount of bile increases again, possibly even above the normal. These statements made it appear probable that the cholesterol of the red corpuscles destroyed would appear in the bile, along with the bile colouring matter. By careful measurements of the quantities of bile produced in dogs in which permanent fistulæ had been established in a satisfactory manner, and by estimations of cholesterol in the bile, by methods which do not seem to us open to objection, before and after the administration of toluylene diamine, Kosumoto showed that this was the case. The conclusion seems to be justifiable that a part at any rate of the cholesterol of the bile arises from the *débris* of the normal destruction of red blood corpuscles in the liver.

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* 'Pflüg. Archiv f. d. Ges. Physiol.,' 1898, vol. 73, p. 595.

† 'Bioch. Zeit.,' 1908, vol. 13, p. 354.

‡ 'Arch. f. experim. Pathol. u. Pharmak.,' 1881, vol. 14, pp. 231, 422.

§ 'Zeit. f. klin. Med.,' vol. 6.

On the other hand, the percentage of cholesterol in the fistula bile of dogs does not appear, according to the investigations of Goodman,* to depend upon the cholesterol content of the food taken by the animals, a result in accordance with the work of previous observers. Goodman made the interesting observation that with a diet of 725 grammes of coagulated white of egg, which contains little or no cholesterol, he was able, during five days, to collect 477 grammes of bile containing 0·208 gramme of cholesterol, whilst with a diet of 488 grammes of calves' brain, which is very rich in cholesterol, he obtained in four days 367 grammes of bile containing 0·145 gramme of cholesterol, so that the percentage content in cholesterol of the bile excreted in these two diets was 0·0436 in the case of the white of egg, and 0·0395 in that of brain. It seems probable, therefore, that the cholesterol of the bile is not derived directly from the food, and some of it at any rate is the result of the elimination of the cholesterol of dead blood corpuscles, and possibly also the *débris* of other tissues, by the liver. On the other hand, this cholesterol can in no sense be regarded as a waste product, as we have shown in previous papers† that herbivorous animals do not excrete cholesterol or any recognisable derivative of that body in their fæces, although their bile contains considerable quantities, in the case of the cow for example 0·07 per cent.‡ Further, in the case of the dog any cholesterol found in the fæces can be entirely accounted for by the cholesterol contained in the food taken. In experiments in which cholesterol free food was given no cholesterol was found; thus a dog fed for 31 days on oatmeal and water passed no more than 0·1 gramme of impure cholesterol in the fæces.

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* 'Hofmeister Beit.,' 1907, vol. 9, p. 91.

† 'Roy. Soc. Proc.,' B, vol. 80, p. 12.

‡ 'Journal of Physiol., Proc.,' 1907, vol. 36, p. ix.

From these observations we were led to the conclusion that the cholesterol of the bile must either be destroyed, or absorbed, along with the bile salts in the intestine, and taken into the blood stream.

The latter hypothesis is more in accordance with the great stability of the cholesterol molecule, and is supported by the observation of Pribram* that emulsions of cholesterol with olive oil, when injected into the stomach of the rabbit, cause an increase in the cholesterol content of the blood.

From a consideration of these facts we have been led to put forward with regard to the origin and destiny of cholesterol the following working hypothesis:—

1. Cholesterol is a constant constituent of all cells, and when these cells are broken down in the life process the cholesterol is not excreted as a waste product, but is utilised in the formation of new cells.

2. A function of the liver is to break down dead cells, *e.g.*, blood corpuscles, and to eliminate their cholesterol in the bile.

3. After the bile has been poured into the intestine in the process of digestion the cholesterol is reabsorbed, probably in the form of esters, along with the bile salts, and carried by the blood to the various centres and tissues for re-incorporation into the constitution of new cells.

The question arises whether the excretion and subsequent absorption of the cholesterol of the body form a regular and exact cycle, or whether there is any wastage of cholesterol which would require to be made up by the animal either by actual synthesis in the body from simpler substances or by the utilisation of that taken in the food. With reference to the wastage of cholesterol it must be pointed out that not inconsiderable quantities are excreted through the skin; the sweat and sebum of men have been shown to contain cholesterol, and in the case of the sheep the sweat, being absorbed by the wool, enables us to demonstrate the presence of large quantities of cholesterol and isocholesterol. How is the wastage made up? Considering the remarkable chemical nature of cholesterol it would appear less probable that it is synthesised by the animal than that the loss is made up by the absorption of cholesterol obtained from the food. In the case of carnivora the cholesterol is contained in their food as such, and might easily be utilised. On the other hand, the food of the herbivora contains no cholesterol, but instead the closely related phytosterols, and the question arises whether this closely allied substance can be utilised by the animal.

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* 'Bioch. Zeit.,' 1906, vol. 1, p. 413.

If the wastage is made up in this way we should expect to find variations in the cholesterol content of the blood according as the food was free from, or rich in, cholesterol. If, further, it were able to take in from its richer diets more cholesterol than was at the moment required, storing it up in the intervals of feeding to replace loss we should expect considerable variations in the cholesterol content of the plasma with the variety of the food. If, however, the animal only takes up what is required to supply immediate waste we should not expect more than a slight variation on different diets, and this variation might easily be entirely masked owing to the different quantities of bile caused to flow into the intestine under the influence of foodstuffs of different kinds. With regard to the mechanism of the absorption of cholesterol in the intestine it would seem probable that it is first esterified, being converted into the oleic and palmitic esters. These compounds, which are stated to possess the property of forming with aqueous fluids lanoline-like emulsions,* were found by Hürthle to be constantly present in the blood plasma of various animals.†

With a view to testing the validity of these considerations we planned a series of experiments, the first instalment of which is described in the present paper.

Experiments to Ascertain whether Cholesterol is Absorbed by Herbivorous Animals when given with their Food.

The animal selected for this investigation was the rabbit. Preliminary experiments showed that the bulk of the phytosterols of bran can be extracted along with the fat by means of ether, without altering the appearance of the bran and without impairing its feeding value, except for the elimination of the fat. It was found that rabbits could be kept for long periods on this diet without apparent injury to health, and often without much loss in weight, though individuals varied considerably in this respect.

The general method adopted in these experiments was as follows:—A rabbit was fed for several days previous to the commencement of an experiment on extracted bran. It was then given each morning 0·25 gramme of cholesterol mixed with a few grammes of extracted bran, care being taken to see that the animal ate the whole. After eating the cholesterol-bran mixture the animal was allowed during the rest of the day as much extracted bran as it would eat. This procedure was followed until the animal had eaten 2 grammes of cholesterol, after which it was fed on extracted bran only for three days in order to sweep all cholesterol from its gut. The fæces during the experiment were carefully collected, dried in the oven at 80° to 90° and weighed. The fæces were then extracted with ether in a Soxhlet's apparatus for a week or ten days. The ethereal solution was saponified according to the method described in our former paper* by means of sodium ethylate. The precipitated soap was filtered off and washed thoroughly with ether. The ethereal filtrate and washings were freed from excess of alkali and alcohol by repeatedly washing with water, dried, and the ether distilled off. The dry residue was weighed and then fractionally crystallised from alcohol until no further crystalline matter could be obtained. The oily residues were then dried, dissolved in pyridine, and treated with excess of benzoyl chloride and after standing over night poured into water. The precipitated matter was filtered, taken up with ether, dried, and the ether again evaporated. The residue was boiled with a little alcohol and any cholesterol that had remained in the oily residue was thus obtained in the form of the highly insoluble benzoate.

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* Ivor Bang, 'Ergebnisse d. Physiologie,' vol. 2, p. 180.

† 'Zeit. Physiol. Chemie,' vol. 21, p. 331.

Experiment I.—In order to ascertain whether it was possible to extract the whole of the cholesterol from fæces by the method used, 2 grammes of cholesterol were ground up with moistened fæces that had already been extracted, in the proportions usually found. The mixture was dried and subjected to the whole process detailed above, when 2·098 grammes of slightly brown-coloured cholesterol were obtained—a quantitative recovery. As the cholesterol recovered from natural fæces is often highly coloured and can only be readily purified by treatment with animal charcoal, it was also desirable to ascertain what loss occurred under the conditions usually followed. The two grammes of recovered cholesterol were therefore dissolved in about 50 c.c. of alcohol, boiled with about half the weight of animal charcoal, and filtered by means of a hot funnel. The charcoal was then washed with hot alcohol. On evaporating the alcohol and crystallising the cholesterol 1·8 grammes were recovered. The loss due to boiling with charcoal was therefore about 10 per cent.

Experiment II.—In order to ascertain (1) how far it was possible to extract the phytosterol from bran by simply extracting with ether for several days; (2) whether any cholesterol could be detected in the fæces, after phytosterol had been eliminated as far as possible from the diet; and (3) how far the quantity of the oily unsaponifiable matter was affected by the use of ether extracted food; and (4) whether an animal could be kept in a healthy state on a prolonged, ether extracted diet, a rabbit weighing 2·4 kilogrammes was fed from December 30, 1907, to January 15, 1908, inclusive on extracted bran, moistened with a little water. The fæces were collected from January 2nd to the 15th inclusive and weighed, after drying, 462 grammes. During this period the animal was given 1·12 kilogrammes of extracted bran, most of which it ate with apparent satisfaction. On January 15th its weight was 2·4 kilogrammes and during the period of the experiment varied from 2·4 to 2·5 kilogrammes.

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* 'Roy. Soc. Proc.,' B, vol. 80, p. 212.

The fæces were extracted with ether and the ethereal solution saponified in the manner described; 1·215 grammes of dry unsaponifiable matter was obtained as a viscid stiff oil. This dissolved in a small quantity (20 c.c.) of absolute alcohol, with the exception of 0·05 gramme of insoluble tar. On adding enough water to make the alcohol about 85 to 90 per cent. strength and allowing to stand, some crystalline matter separated and, after filtration and drying, was obtained in the form of dark brown greasy crystals weighing 0·4 gramme.

This brown crystalline matter was decolorised by animal charcoal and carefully recrystallised fractionally from alcohol. The first and main crop melted at 135° to 136°, and under the microscope had the form of transparent long hexagonal plates. It was identical with the "phytosterol" which we had isolated from the bran. The mother liquors yielded a small crop of crystals which under the microscope were more indeterminate, being grouped in masses and stars. On recrystallisation, however, long hexagonal crystals were again obtained melting at 133° to 135°. *No trace of cholesterol could be discovered.*

Experiment III.—Immediately on the close of the above experiment the same rabbit was fed from January 16 to January 25 inclusive, on 80 grammes of extracted bran with 0·25 gramme of cholesterol per day, except on two days on which no cholesterol was given. It received therefore in this period 2 grammes of cholesterol. It was then fed for three days on extracted bran alone, and the fæces were collected during the whole period. After drying, the fæces weighed 497 grammes. The weight of the animal remained constant all through the experiment.

The ethereal extract was reddish in colour, with a green fluorescence, which was just as marked after saponification; 2·56 grammes of dry unsaponifiable matter were obtained. This dissolved in absolute alcohol leaving 0·06 gramme of insoluble tar. Water was then added to reduce the alcohol to 90 per cent., and on standing, brown crystalline matter was deposited weighing 1·14 grammes. On further standing another crop weighing 0·28 gramme was obtained. The mother liquors were then evaporated to dryness, dissolved in pyridine and treated with benzoyl chloride; 0·1035 gramme of crude benzoate of cholesterol was obtained. The total weight of crude cholesterol was thus 1·5 grammes. The two crops of cholesterol were decolorised by animal charcoal and recrystallised from alcohol. The larger crop melted at 139° and was practically pure cholesterol, but the smaller crop softened at about 125° and melted at 135°. Under the microscope the latter appeared to consist mainly of cholesterol, but was contaminated with phytosterol. The benzoate after recrystallisation melted at 144° to 145° to a turbid liquid which cleared at 180° and on cooling showed the characteristic play of colour in a well-marked manner. About 1 gramme of purified cholesterol was in this way obtained. Evidently therefore the rabbit had absorbed between 0·5 and 1 gramme of cholesterol during the time of the experiment. The animal remained in good health for some weeks afterwards, its weight remaining constant, when it was killed for another purpose.

Experiment IV.—The rabbit used in this experiment weighed 1·7 kilogrammes, and was very thin. It was fed for the three days prior to the commencement of the experiment on extracted bran; it was then given 0·25 gramme of cholesterol and 60 to 70 grammes of extracted bran daily for eight days, and extracted bran alone for three more days. Care was taken that the animal took the whole of the cholesterol, but it wasted a good deal of the bran. The animal lost weight during the whole experiment, and died the day after, when its weight was 1·3 kilogrammes. A *post-mortem* examination showed that the animal was very thin, and in poor condition. The intestine was filled with a watery fluid, the liver very dark in colour, and the stomach dilated with gas. It may be noted that during this experiment the weather was very cold, and for a few days the heating apparatus was out of order, so that possibly this may have had something to do with the death of the animal, which was not in the best of condition at the start.

Three hundred and twelve grammes of dry fæces were obtained. The ethereal extract was pale yellow in colour, and on evaporation gave 1·46 grammes of unsaponifiable matter as a greasy brown solid. This dissolved in 85 to 90 per cent. alcohol, with the exception of a small amount of tar which was insoluble in absolute alcohol, though soluble in ether. On standing, the solution deposited 0·79 gramme of brown crystalline matter. A further crop, weighing 0·32 gramme, was obtained on long standing. This was more granular in appearance, and rather sticky. The mother liquors were evaporated to dryness and benzoylated in pyridine solution, but no matter difficultly soluble in alcohol could be isolated.

The two crops were decolorised by animal charcoal and recrystallised. The first deposit melted at 142° to 143°, and under the microscope was seen to consist of practically pure cholesterol. The second crop of crystals melted at 141° to 142°, but a microscopic examination showed that it contained some phytosterol, as the shape of the crystals deviated from those of cholesterol in the well-known manner occasioned by traces of phytosterol. A final crop melted at 138° to 139°, and contained a larger amount of phytosterol. In this experiment it is evident that at least 1 gramme of cholesterol was absorbed.

Experiment V.—A rabbit, weighing 1·7 kilogrammes, was treated exactly as in Experiment IV. At the end it weighed 1·6 kilogrammes, and appeared to be in good health; 277 grammes of dry fæces were obtained, which were extracted for 14 days in a Soxhlet's apparatus with ether. The ethereal solution was deep red, with a strong green fluorescence. The dry unsaponifiable matter weighed 2·275 grammes, and was dissolved in 70 c.c. of hot alcohol, leaving 0·05 gramme of insoluble tar. After adding 6 c.c. of water to the hot alcohol solution, 1·39 grammes of red crystalline matter were deposited, from which 1·125 grammes of fairly pure cholesterol were obtained. The mother liquors, on standing, deposited a small quantity of crystalline matter mixed with oil, from which, after boiling in alcoholic solution with animal charcoal, 0·11 gramme of white crystals was isolated. All the mother liquors were evaporated and benzoylated, but only a small trace of difficultly soluble matter resulted. The weight of cholesterol recovered was therefore 1·23 to 1·4 grammes, which, however, would contain any phytosterol present.

Experiment VI.—A rabbit, weighing 1·7 kilogrammes, was fed on extracted bran and cholesterol as in the previous experiments. The weight remained practically constant, but after the experiment, on being put on ordinary diet, it began to lose weight, and died on the eighth day. The weight of the dead animal was 1·4 kilogrammes, but a *post-mortem* examination revealed nothing abnormal.

The weight of dry fæces collected was 283 grammes, and they were extracted for 14 days. The ethereal extract was pale yellow in colour, but without any fluorescence. The unsaponifiable matter weighed 3·13 grammes, but was not free from calcium chloride. It was boiled with absolute alcohol, when 0·145 gramme of insoluble matter was left. The alcoholic solution after dilution with water until the strength was 85 to 90 per cent., gave 1·47 grammes of not very coloured crystalline matter. The mother liquors yielded between 0·1 and 0·2 gramme of impure crystalline matter. After recrystallisation of the whole from the least amount of 90 per cent. alcohol, the melting point was 135°.

The results of these experiments are summarised in Table I.

It is clear from these experiments that (1) cholesterol is not excreted by rabbits unless they are fed on it, which is in agreement with our previously

Table I.

Exp.	Duration of experiment, in days.	Weight of rabbit at beginning, in kilogrammes.	Weight at end.	Weight of cholesterol given.	Weight of bran, in kilogrammes.	Weight of dry fæces.	Total unsaponifiable matter dry, in grammes.	Weight of crude cholesterol recovered, including phytosterol.
II.	14	2·4	2·4	—	1·12	462	1·215	0·4 of phytosterol
III.	11	2·4	2·4	2	1·01	497	2·56	1·5
IV.	11	1·7	1·3	2	0·67	312	1·46	1·11
V.	11	1·7	1·6	2	0·73	277	2·27	1·23—1·4
VI.	11	1·7	1·7	2	0·73	283	3·13	1·4—1·6

published results; (2) that when cholesterol is administered with the food a portion of it is absorbed, in our experiments about 50 per cent. It is also clear that vegetable food such as bran or grass can be freed from fat and phytosterols by extraction with ether without impairing its feeding value.

Is Cholesterol Absorbed from the Food by Carnivorous Animals?

In our former paper on the excretion of cholesterol by the dog* we described experiments which, although they were carried out primarily for the purpose of showing that the cholesterol content of the fæces was a function of the cholesterol in the food taken, may yet be considered as evidence for the absorption of some cholesterol from the food in this animal. But such evidence cannot be regarded as of the same conclusive nature as that afforded by the experiments on the rabbit, because the cholesterol content of the various foodstuffs given is not known with any degree of certainty. The estimations of Dormeyer†, for the cholesterol content of dry muscle (0·23 per cent.), are perhaps as satisfactory as any, though they probably err, if anything, in being too high. However, if we take such a value and apply it to our own data we arrive at such results as the following. In one experiment a dog in 20 days ate 7470 grammes of cooked beef and mutton, the percentage of solids in which we found to be about forty. Allowing for the fact that the meat did not consist entirely of muscle and for variations of other kinds, we may perhaps halve Dormeyer's value in this case. On this assumption, then, the animal consumed 3·4 grammes of cholesterol, whereas 0·8 gramme only was found in the fæces, so that a disappearance of about 2·5 grammes of cholesterol is indicated.

* 'Roy. Soc. Proc.,' B, vol. 80, p. 227.

† 'Pflüg. Archiv,' vol. 61, p. 341.

In another experiment the animal ate 6758 grammes of horseflesh in 17 days. Making the calculation as before, we find that the dog may have eaten 4 grammes of cholesterol, whereas only 1 gramme was discovered, pointing again to an absorption of possibly 3 grammes.

We did not institute any further experiments with the dog on these lines on account of the numerous uncertainties involved. We have, however, lately been successful in discovering a cholesterol-free diet comparable with extracted bran on which cats can be fed, and have begun an elaborate series of experiments on the question which we hope to communicate in the near future. Preliminary experiments on cats showed us that, as in the case of dogs, the cholesterol of animal food is passed in the fæces as such, but that on a brain diet these animals also convert the cholesterol into coprosterol. The two following experiments may be quoted here as bearing on this interesting point.

Experiment VII.—A cat weighing 2·8 kilogrammes was fed on raw sheep's brain for 14 days. The fæces were somewhat liquid, and dried at 100° to a soft, sticky, glue-like mass, which was ground up with excess of sand before extracting with ether. Weight of dry fæces, 245 grammes. During the period of diet the animal lost 0·5 kilogramme in weight, which loss, however, it subsequently regained on ordinary diet; 28 grammes of unsaponifiable matter, in the form of a dark red viscid oil were obtained. By fractional crystallisation from acetone between 18 and 19 grammes of brown crystalline matter were separated. This consisted of coprosterol, and after further purification from dilute alcohol 12 grammes of perfectly pure coprosterol were obtained. This melted at 99° to 100° C., and had a specific rotatory power (in chloroform) $[\alpha]_D^{16} = +20^\circ{\cdot}4$.

On a diet of raw sheep's brain, therefore, the cat changes cholesterol into coprosterol in the same way as we showed was the case with the dog.* If we assume that sheep's brain contains 2 per cent. of cholesterol, our cat should have consumed in the 14 days some 34 grammes of cholesterol, which would correspond with an absorption of about 15 grammes, or 1 gramme per day. Owing to the difficulty of crystallising coprosterol completely from the oily matter with which it is mixed in the unsaponifiable residue, one cannot claim for this estimation any high degree of certainty. It is, however, significant that the total weight of unsaponifiable matter obtained was less than the total cholesterol that should have been consumed, and there can be no doubt that the extraction of the fæces by ether was a very thorough one, as it was allowed to go on for 14 days, the ether distilling over during the day and being allowed to soak on the material during the night.

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* 'Roy. Soc. Proc.,' B, vol. 80, p. 227.

Experiment VIII.—In this experiment the sheep's brain was lightly fried in its own oil before being given, in order to ascertain whether the cooking process had any influence on the conversion of the cholesterol to coprosterol. The cat selected weighed 2·9 kilogrammes, and during the feeding period of 14 days lost 0·4 kilogramme in weight; 1913 grammes of brain (weighed uncooked) were consumed, and 365 grammes of dried fæces obtained. These were of very much the same constituency as before, and were treated in a similar manner; 23 grammes of unsaponifiable matter in the form of a red oil were obtained. This proved very difficult to purify, owing to the tarry, sticky oils present, and we were only able to isolate 7·4 grammes of white crystalline matter, which proved to be a mixture of cholesterol and coprosterol. The cholesterol taken (on the above assumption) should therefore be 38 grammes, the total unsaponifiable matter being only 23 grammes. The cooking and consequent partial sterilisation of the brain seem, therefore, to have interfered with the conversion of the cholesterol into coprosterol.

EXPERIMENTS TO ASCERTAIN WHETHER ANY OF THE CHOLESTEROL WHICH DISAPPEARS FROM THE FOOD CAN BE FOUND IN THE BLOOD.

Herbivorous Animals.

Pribram[*] has stated that on administering cholesterol to rabbits *per os*, an increased percentage of this substance could be found in the blood. His method consisted in injecting into the stomach of the animal an emulsion of cholesterol, cholesterol oleate, or cholesterol palmitate, made up with olive oil. After some hours the animal was killed, and the cholesterol in the blood determined in the usual way. But from the point of view of proving that cholesterol taken by the mouth can be absorbed into the blood stream these experiments seem to us for several reasons by no means conclusive. In the first place, the rabbits studied were far from being under normal conditions of diet. A comparatively large dose of oil was put into the stomachs of animals who are not accustomed to take or assimilate fats in this form. Pribram mentions that the oil passed into the blood as the serum became opalescent, and it seems to us not improbable that, with a quantity of oil in the stomach which cannot be assimilated in the ordinary way, some of the oil might percolate, if one may use the term, into the blood, carrying the cholesterol with it. The supposed increase, therefore, found in the blood might not unreasonably be due to a mechanical rather than to a metabolic process. But a further consideration of the data given by Pribram led us to doubt whether they could be considered as showing that there was an increased percentage of cholesterol.

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[*] 'Bioch. Zeit.,' vol. 1, p. 414.

The standard of comparison employed by him was the cholesterol content of the blood of a starving rabbit, which again is an abnormal case, since conceivably some cholesterol might disappear from the blood during starvation, and if this is so it is probably a variable standard, as different animals would vary in the rate at which the cholesterol was removed from their blood. Again, the quantities of cholesterol isolated and weighed were extremely small, and were admittedly not pure. No melting points or other constants were given, and we are consequently left uncertain as to whether the matter weighed was cholesterol in a more or less pure state, or whether it was largely composed of crude unsaponifiable matter, or whether it contained any cholesterol at all. As the percentages found were so small, a very slight variation in the amount of impurity present would invalidate and even entirely reverse the conclusions deduced by the author. In a second series of experiments, however, which bring out the increased inhibitory power of the serum of the cholesterol-fed rabbit towards the hæmolytic effect of saponin, the results are more satisfactory, and certainly speak for an assumption of cholesterol by the blood. But our objection to the method of dosage adopted still holds, and the number of hæmolytic experiments carried out was too few. Our own experiments in comparing the action of sera in this respect have shown us that there is a very considerable variation in the action of the sera of individual animals of the same species, when treated under precisely similar conditions. The discussion of this point, however, we leave for the present, as we hope to make it the subject of a communication in the near future.

In the experiments we have carried out to ascertain the fate of the cholesterol which disappears from the food we have endeavoured to avoid the difficulties pointed out in the preceding paragraph in the following way:—

1. We adopted as a standard of comparison a rabbit which was fed for a long period on a cholesterol-free diet, viz., bran thoroughly extracted with ether. The blood of such an animal was compared with that from others which had been fed in an exactly similar way as to times and quantities of extracted bran, but whose food contained in addition a measured daily quantity of cholesterol. In this way we had two rabbits feeding on practically the same diet under the same conditions, and accordingly the chance of variations, especially in the bile flow, due to differences in the food taken was reduced to a minimum, and the only variation likely to interfere was that due to the individual peculiarities of different rabbits which are inevitable in such experiments.

2. With regard to the estimation of cholesterol in the tissues and the

blood, we must again emphasise the fact that the weight of crude unsaponifiable matter obtained from them gives little or no idea of their cholesterol content. In our experience, the ether extract of animal tissues always contains relatively large quantities of low melting oily or resinous bodies, which may prove to be of very considerable importance in biochemistry, but they are non-crystalline and cannot be considered as cholesterol. Furthermore, their amount is very variable, so that even for purposes of comparison the weights of crude unsaponifiable matter are useless. Our own procedure was as follows: The blood, if dried in the ordinary way, becomes a very hard horny mass which even if powdered is difficult to extract. We therefore mixed the blood after whipping to prevent coagulation with sand and plaster of Paris in sufficient quantity to form a friable mass. This was ground up and extracted for 14 to 30 days, the heating being stopped during the night so that the ether might thoroughly soak into the material. The extract was saponified in the manner we have previously described, and the non-saponifiable residue converted directly to benzoate in pyridine solution, the cholesterol being thus weighed in the form of cholesterol benzoate.

Experiment IX.—Rabbit A. A rabbit, weighing 2·8 kilogrammes, was fed for 21 days with 70 to 80 grammes of extracted bran per day, and then killed 24 hours after the last supply of food had been placed in the cage. A *post-mortem* examination showed that the stomach still contained some food. The animal during this period lost 0·3 kilogramme in weight. The weight of blood obtained was 73 grammes, from which 0·14 gramme of unsaponifiable matter in the form of a stiff oil was obtained. The quantity of cholesterol contained in this was so small that it was not found possible to isolate any in a pure state.

Experiment X.—Rabbit B. This rabbit, weighing 2·2 kilogrammes, was fed for three days on extracted bran, then during 10 days on 540 grammes of extracted bran, mixed with 2¼ grammes of cholesterol, care being taken that the whole of the cholesterol was eaten. The weight of the animal remained unaltered during this period, and it was killed 24 hours after the last meal had been placed in the cage. The blood obtained weighed 71 grammes and yielded 0·29 gramme of crude unsaponifiable matter, from which 0·0375 gramme of pure cholesterol benzoate was obtained. The specimen, which was actually weighed without further crystallisation, melted at 142° to 143° to a turbid liquid which became clear at 170°, and on cooling showed the characteristic play of colours. This quantity corresponds to a yield of 0·0295 gramme of cholesterol or 0·0415 per cent.

Experiment XI.—Rabbit C. In order to compare the cholesterol content of an animal fed on a normal diet which contained phytosterol but not cholesterol, a rabbit weighing 2·8 kilogrammes was fed on a liberal mixed diet of cabbage, oats, and bran for a month, and killed after 24 hours as in previous experiments. The blood obtained weighed 75 grammes, which yielded 0·117 gramme of unsaponifiable matter as a brown oil mixed with crystalline material. After treating with benzoyl chloride in the usual way 0·028 gramme of greasy crystals was obtained, which were obviously not pure. Under the microscope these appeared as star-shaped aggregates of needles mixed with indeterminate matter, but no typical crystals of cholesterol benzoate were observed, and the substance could not be further purified. It is obvious that there is not sufficient cholesterol in the blood of a single rabbit, when fed on a non-cholesterol, or on a normal diet, for an accurate quantitative estimation. We therefore fed six rabbits, weighing 1·5, 1·7, 1·4, 1·9, 1·5, 1·9 kilogrammes respectively, on a liberal diet of oats, bran, and greens for a week. They were then killed and the total blood taken. This weighed 500 grammes. On treatment in the usual way 0·464 gramme of unsaponifiable matter as a brown, slightly greasy solid was obtained. This was crystallised from alcohol; the first crops, weighing respectively 0·091 and 0·049 gramme, consisted, as a microscopic examination showed, mainly of cholesterol, plate-like crystals of which were mixed with minute spherules of some other substance. These crystals were dried and treated in pyridine solution with benzoyl chloride. All the mother liquors remaining were evaporated to dryness and treated with pyridine and benzoyl chloride. The benzoate found was recrystallised from a measured quantity of absolute alcohol; 0·1523 gramme of pure cholesterol benzoate in all was thus obtained, which, without further purification, melted correctly, and gave the colour play of cholesterol benzoate; 0·1199 gramme of cholesterol was thus obtained from the blood of six rabbits or 0·024 per cent.

It is clear from these experiments that Pribram was correct in his conclusions, and that cholesterol can be absorbed from the intestines into the blood of the animal, since in the case of the rabbits which had been fed on cholesterol we were easily able to prepare and weigh pure cholesterol benzoate, whereas in the case of a rabbit fed on extracted diet, or on normal diet, the quantity was so small that we were unable to obtain any cholesterol from it in a pure state. In order to get a precise figure for the cholesterol content of the blood of the rabbit under normal conditions we were obliged to deal with the blood of six rabbits.

In the case of rabbits A, B, and C we made estimations of the cholesterol contained in the brain and spinal cord, and in the rest of the animal respectively. We can, however, draw no conclusions from the results of the experiments, but we think the actual determinations are of sufficient interest and accuracy to be placed on record. The method we adopted for the brain and cord was to mix with plaster of Paris in a mortar, and, after the mass has hardened, to powder it thoroughly. This was then thoroughly extracted with ether (21 days) and the extract treated in the usual way. The rest of the rabbit carcase (including the fur) was finely minced in a machine, mixed with plaster of Paris, and again passed through the machine. After it had set to a dry mass it was ground up in a mortar with coarse sand and plaster of Paris, and extracted for three weeks with ether. The results are collected together in the following table :—

Table II.

	Weight in grammes.	Unsaponifiable matter.	Pure cholesterol found.	Cholesterol per cent.
Rabbit A, weight 2·5 kilos., fed on extracted bran for 20 days.				
Blood	73	0·14	(trace)	—
Brain and spinal cord	14·3	0·68	0·427	3·0
Rest of rabbit	2413	3·75	2·168	0·09
Rabbit B, weight 2·2 kilos., fed on extracted bran + 2¼ grammes of cholesterol for 10 days.				
Blood	71	0·29	0·0295	0·0415
Brain and spinal cord	13·31	1·31	(lost)	—
Rest of rabbit	2116	3·50	1·908	0·09
Rabbit C, weight 2·8 kilos., fed on a mixed diet of cabbage, oats, and bran for 1 month.				
Blood	75	0·117	(trace)	—
Brain and spinal cord	17·5	0·776	0·5225	3·0
Rest of rabbit	2708	5·11	2·716	0·10
Six rabbits, fed on above mixed diet.				
Blood	500	0·464	0·1199	0·024

Experiments to ascertain whether the Cholesterol Content of the Blood can be Correlated with Variation in the Cholesterol Content of the Food in Carnivorous Animals.

A. *Experiments in which the Animals were killed two to four hours after a Meal.*

Experiment XII.—A dog, weighing 7·36 kilogrammes, was fed for 10 days on a daily ration of 200 to 300 grammes of bread, the whites of two eggs, and a teaspoonful of cream, the whole being moistened with a solution of Liebig's extract of beef, and lightly fried. The animal was killed three and a half hours after the last meal. The blood weighed 480 grammes, and from this 0·687 gramme of unsaponifiable matter was obtained. This was at once benzoylated in pyridine solution, and a total crop of cholesterol benzoate weighing 0·4865 gramme was separated. This corresponds to 0·3892 gramme, or 0·0811 per cent. of cholesterol.

A *post-mortem* examination showed that the stomach was practically empty. The gall-bladder contained 5·2525 grammes of bile which on evaporation yielded 0·892 gramme of solid matter. From this 0·002 gramme of cholesterol benzoate was obtained, or 0·18 per cent. of cholesterol (calculated on the dry solids).

Experiment XIII.—A dog, weighing 8·13 kilogrammes, was fed for nine days on a daily ration of 250 grammes of raw brain. At first it did not take kindly to this food, but in the last few days it consumed the whole of the brain given. The animal was killed two hours after a meal. The weight of blood obtained was 430 grammes, which yielded 0·74 gramme of unsaponifiable residue. This was benzoylated directly in pyridine solution, and 0·484 gramme of pure cholesterol benzoate was obtained, melting correctly. This corresponds to 0·3872 gramme, or 0·09 per cent. of cholesterol.

A *post-mortem* examination showed that the stomach contained some undigested food. The gall-bladder contained 2·06 grammes of bile of a pale yellow colour, and left 0·2245 gramme of solid matter. From this 0·004 gramme of unsaponifiable matter was obtained, but only a trace of benzoate could be separated from it.

Experiment XIV.—A dog, weighing 9·77 kilogrammes, was fed for nine days on a daily ration of 200 grammes of dry oatmeal made into porridge with water. The animal ate about half the last meal only, and was killed four hours afterwards. The blood weighed 680 grammes and yielded 0·91 gramme of unsaponifiable matter. This, on benzoylation in pyridine solution, gave 0·6775 gramme of pure cholesterol benzoate, corresponding to 0·542 gramme, or 0·0797 per cent. of cholesterol in the blood.

A *post-mortem* examination showed that the stomach contained a quantity of undigested food. The gall-bladder contained 6·36 grammes of bile which, on evaporation, yielded 1·1065 grammes of solid residue. From this only 0·001 gramme of cholesterol benzoate could be obtained, corresponding to 0·07 per cent. of cholesterol.

Experiment XV.—A dog weighing 8·5 kilogrammes was fed for six days on a daily ration of 200 to 300 grammes of brain, mixed with some bread. The animal was killed four hours after the last meal. The blood was unfortunately lost. The gall-bladder contained 702 grammes of bile which, on evaporation, yielded 1·3947 grammes of solid residue. From this, 0·0028 gramme of cholesterol benzoate was obtained, or 0·16 per cent. of cholesterol.

B. *Experiments in which the Animals were killed 24 hours after a Meal.*

Experiment XVI.—A dog weighing 7·8 kilogrammes was fed for 7 days on porridge made by boiling about 100 grammes of oatmeal with water, daily. It was killed 24 hours after the last meal. The quantity of blood obtained was 524 grammes, from which 0·9845 gramme of greasy unsaponifiable matter was obtained. The first crop of crystals from alcohol weighed 0·2915 gramme, and a microscopic examination showed that these consisted of practically pure cholesterol. The residues were benzoylated in pyridine solution and yielded 0·1795 gramme of crystals, which melted correctly and gave the characteristic colour play of cholesterol benzoate. The total cholesterol obtained was therefore 0·435 gramme or 0·083 per cent.

A *post-mortem* examination showed that the stomach was quite empty. The gall-bladder was distended and contained 4·55 grammes of bile, which, after drying at 100° C., left 0·8257 gramme of solid matter. We attempted to estimate the cholesterol in this quantitatively, but the amount was too small for an accurate determination. However, 0·0025 gramme of cholesterol benzoate was isolated, or 0·24 per cent. of the total solids.

Experiment XVII.—A dog weighing 9·1 kilogrammes was fed for 14 days on raw brain. It was given 300 to 500 grammes of fresh brain per day, but the animal did not take the food very well and often left a portion uneaten. On the last day of the period the dog developed feverish symptoms and was killed 24 hours after the last meal. The quantity of blood obtained was 507 grammes and yielded 1·43 grammes of unsaponifiable matter. This was not completely soluble in alcohol. The first crop of crystals weighed 0·5075 gramme and melted at 145° C. A second crop weighed 0·0785 gramme and melted at 144° C. From the residues on benzoylation 0·2115 gramme of cholesterol benzoate, melting at 145°, was isolated. The total cholesterol obtained was therefore 0·7526 gramme, or 0·1486 per cent.

A *post-mortem* examination showed that the stomach was empty and distended with gas, and the lungs appeared to be congested. The gall-bladder contained 0·844 gramme of bile, which was very thick and stringy, and contained 0·1617 gramme of solid matter. From this 0·0025 gramme of cholesterol benzoate was obtained, or 1·2 per cent. of the total solids.

Experiment XVIII.—A large dog weighing 19·2 kilogrammes was fed for 24 days on raw brain together with a little bread. This animal took the food readily and consumed daily from 500 to 800 grammes of brain, and at the end of the period was in good health. It was killed 24 hours after a full meal. The weight of blood obtained was 1140 grammes, which yielded 1·5 grammes of unsaponifiable matter. On crystallisation from alcohol a first crop, weighing 0·304 gramme, melting at 144° to 145°, was obtained, and a second weighing 0·184 gramme and melting at the same temperature. The mother liquors and residues, on benzoylation, gave 0·41 gramme of cholesterol benzoate. The total cholesterol obtained was therefore 0·816 gramme, or 0·072 per cent.

A *post-mortem* examination showed that the stomach was empty. The gall-bladder contained 15·82 grammes of bile, which gave 3·947 grammes of solid matter, and from this 0·017 gramme of cholesterol benzoate was obtained, or 0·34 per cent. of cholesterol.

Experiment XIX.—A dog weighing 13·4 kilogrammes was fed for 10 days on a diet practically free from cholesterol. The daily ration consisted of 250 to 300 grammes of bread, the whites of two eggs, two teaspoonfuls of cream lightly fried together after moistening with a solution of Liebig's extract of beef. The animal continued in good health and was killed 24 hours after the last meal. The blood weighed 760 grammes and yielded 1·326 grammes of unsaponifiable residue. On crystallising from alcohol the first crop weighed 0·4615 gramme and melted at 145°, the second weighed 0·2135 gramme and the same melting point. After benzoylation, the residues yielded 0·1728 gramme of cholesterol benzoate, melting at 145°, and showing the colour changes. The total cholesterol obtained was therefore 0·8132 gramme, or 0·107 per cent. A *post-mortem* examination showed that the stomach was empty. The gall-bladder contained 10·29 grammes of bile which, on evaporation, gave 2·632 grammes of solid matter. From this, 0·003 gramme of cholesterol benzoate was obtained, or 0·09 per cent. of cholesterol.

These results are collected together in Table III.

The experiments differ fundamentally from those carried out on the rabbit described in the earlier part of this paper. In the case of these animals a standard diet free from cholesterol and similar bodies, to which measured portions of cholesterol could be added, was available; and as they are practically continuous feeders the bile flow and consequently the cholesterol content of the blood, due to this source, would remain practically constant. In the case of the dogs we had no such cholesterol-free standard diet and were obliged to make use of a number of entirely different foods, differing as far as possible in cholesterol content, though even the non-cholesterol ones contained phytosterol. Little is known concerning the

Table III.

Experiment.	Weight of dog, in kilogrammes.	Diet.	Diet period, in days.	Weight of blood, in grammes.	Weight of cholesterol in the blood.	Percentage of cholesterol in the blood.
A.—Animals killed *two* to *four* hours after a meal. (Terriers.)						
XII.	7·36	Bread, egg-white, cream	10	480	0·3892	0·0811
XIII.	8·13	Raw brain..................	9	430	0·3872	0·0900
XIV.	9·77	Meal	9	680	0·5420	0·0797
B.—Animals killed *twenty-four* hours after a meal.						
XVI.*	7·8	Meal	7	524	0·4350	0·083
XVII.†	9·1	Raw brain..................	14	507	0·7526	0·148
XVIII.‡	19·2	Raw brain + bread	24	1140	0·8160	0·072
XIX.§	12·67	Bread, egg-white, cream	10	760	0·8132	0·107

* Terrier. † Terrier; dog jaundiced.
‡ Retriever dog. § Collie dog.

influence of food on the excretion of bile, but from experiments that have been made by various observers there is good reason to suppose that the nature of the diet would not be without influence. Furthermore, the dog is a discontinuous feeder and the flow of bile into its intestine would be intermittent. Under these circumstances the portion of the floating (as distinguished from the constitutional) cholesterol in the blood due to the reabsorption of the cholesterol of the bile, would not necessarily be strictly comparable in these different cases. As to what would be the limits of such variation, if any, we have no data at present from which to form an opinion, but a variation of the kind suggested might wholly or partially mask any variation due to the cholesterol absorbed from the food, which, at best, would not be great in absolute magnitude. In the first series of experiments (A) in which the animals are fairly comparable in weight and variety, the blood of the dog fed on brain shows a small increase in the percentage of cholesterol in its blood, though it would not be unreasonable to ascribe this to an extra bile flow due to the fatty nature of the diet. In the second series of experiments (B) in which the blood was taken after the digestive process was completed, the percentages found were of much the same order of magnitude as in the first series, with the exception of Experiment XVII. It will be noticed, however, that the percentage in the blood of the retriever dog fed on brain was slightly less than that in the blood of the dogs fed on meal, and bread and egg-white respectively. These animals were, however, very different in weight and were of different varieties.

Little value can be given to the high figure obtained in Experiment XVII, as the animal was ill at the time its blood was taken.

It seems to us very doubtful whether the chemical methods of estimating cholesterol, which we have endeavoured to make as perfect as possible, are sufficiently accurate to enable us to draw definite conclusions without making an enormous number of experiments of this type. We have, however, recently found a material suitable for the food of cats, which can be rendered cholesterol-free, and a series of experiments are in progress to compare the effect on the blood of the addition of cholesterol to such a diet both by chemical analysis and by comparisons of the anti-hæmolytic effect of the sera. The results of these experiments, which we expect to give more definite information on this subject, we hope to make the subject of a communication in the near future.

The expenses in connection with this work were defrayed by means of a grant made by the Government Grant Committee of the Royal Society, for which we take this opportunity of expressing our thanks.

The Origin and Destiny of Cholesterol in the Animal Organism.
Part IV.—*The Cholesterol Contents of Eggs and Chicks.*

By G. W. Ellis and J. A. Gardner, Lecturer on Physiological Chemistry, University of London.

(Communicated by Dr. A. D. Waller, F.R.S. Received January 15,—Read February 11, 1909.)

(From the Physiological Laboratory, South Kensington, University of London.)

In a paper recently communicated to the Royal Society* the hypothesis was advanced that cholesterol is a substance which is strictly conserved in the animal organism. As it is difficult to conceive how a body of the constitution of cholesterol can be synthesised in the organism from proteids, carbohydrate or fat, it was suggested that the waste of cholesterol might be made up from the food taken by the animal. In order to test the correctness of this view we thought that evidence of fundamental importanc might be obtained by comparing the cholesterol content of eggs and newly-hatched chicks, and also by ascertaining whether chicks could be reared and would thrive on food deprived of its cholesterol or phytosterol. In this paper we give an account of our estimations of cholesterol in hens' eggs and newly-hatched chicks.

Method of Estimation.—The weighed egg or chick (including broken shell) was pounded up in a mortar with sand and sufficient plaster of Paris to cause the whole to set after a time to a dry mass. This was powdered and extracted in a Soxhlet's apparatus with ether for about twelve days. The ethereal solution of the extract was saponified in the cold by means of an alcoholic solution of sodium ethylate. After standing overnight the precipitated soap was filtered off and thoroughly washed with ether. The filtrate and washings were repeatedly shaken with water to get rid of alcohol, excess of alkali, traces of soap, etc., dried with calcium chloride and the ether distilled off. The residue was dried at 100° C. and weighed. In the case in which single eggs or chicks were analysed, the dry residue, dissolved in 10 c.c. of pyridine, was mixed with about three times its weight of benzoyl chloride also in solution in 10 c.c. of pyridine. After standing overnight the liquid was poured into water, and the precipitated cholesterol benzoate after drying was boiled with 10 c.c. of absolute alcohol and allowed to stand some hours. The crystals were filtered off, washed with a little absolute alcohol and weighed. In all cases these crystals were colourless, or nearly so, and melted approximately correctly. The filtrate and washings were separately measured, and corrections, which had been ascertained by previous experiments, made for the solubility of cholesterol benzoate. When cholesterol benzoate was crystallised from absolute alcohol the mother liquor at 21° C. contained 0·12 per cent. When, however, ready formed crystals were shaken for a short time with alcohol at 20° C. and filtered, the filtrate was found to contain only 0·04 per cent. When a number of eggs or chicks were analysed together, the unsaponified residue was crystallised from alcohol, and as much pure cholesterol as possible was isolated, melting at 145°—147° C. The mother liquors were then evaporated to dryness, benzoylated in a pyridine solution as described above, and the benzoate weighed. The soaps precipitated on saponification were collected together in two lots, comprising respectively the total amount obtained from all the eggs examined, and the total amount obtained from all the chicks. These were separately mixed with about twice their weight of salt, water added, and after evaporating to dryness were thoroughly extracted with ether. In neither case could any appreciable quantity of cholesterol be isolated.

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* 'Roy. Soc. Proc.,' this vol.

In Table I the analysis of eight eggs is given, and in Table II the analysis of eight chicks.

It is obvious from these figures that no increase in the quantity of cholesterol takes place during the change from ovum to newly-hatched chick, the average percentage of cholesterol in eggs being 0·3827, and in chicks 0·3693, or in terms of the weight of the original eggs 0·3172. The same result follows, no matter whether we take the figures for crude unsaponifiable matter, or those for pure cholesterol.

At first sight it would appear that a loss of cholesterol occurs, but taking into account the facts that the difference between the average percentage of cholesterol in eggs and chicks (column *e*)—0·066—is of much the same order of magnitude as the average deviation from the mean in the two cases, viz., 0·057 for eggs and 0·075 for chicks. That individual eggs differ considerably in the loss in weight which takes place during incubation, that there is no reason to suppose that the proportion of yolk to white in different eggs is very constant, and that the method of estimation of cholesterol does not possess a very high degree of accuracy, it would seem probable that no change in the quantity of cholesterol takes place, and that all the cholesterol of the egg is contained in the newly-hatched chick. In order to obtain a more accurate value for the cholesterol content of eggs and chicks, six eggs and six chicks were analysed together, as the greater the quantity of cholesterol weighed in an analysis the more accurate is the

Table I.—Analysis of Separate Eggs.

No.	Weight of eggs in grammes.	Weight of unsaponifiable matter in grammes.	Weight of cholesterol in grammes.	Percentage of cholesterol.
1	67·33	0·4024	0·2243	0·3331
2	57·57	0·3490	0·1978	0·3436
3	53·32	0·3655	0·2104	0·3946
4	58·48	0·3768	0·2162	0·3697
5	52·70	0·3265	0·1356	0·2563
6	55·40	0·3628	0·2582	0·4661
7	57·30	0·3923	0·2570	0·4485
8	55·45	0·3735	0·2514	0·4534
Total ...	457·55	2·9488	1·7509	0·3832

Table II.—Analysis of Separate Chicks.

No.	Weight of eggs in grammes.	Weight of chicks in grammes.	Weight of unsaponifiable matter in grammes.	Weight of cholesterol in grammes.	Percentage referred to weight of egg.	Cholesterol referred to weight of chick.
	a.	*b.*	*c.*	*d.*	*e.*	*f.*
9	59·80	55·20	0·3490	0·2014	0·3368	0·3649
10	58·14	53·20	0·3130	0·1819	0·3129	0·3419
11	67·55	61·10	0·4415	0·1433	0·2121	0·2345
12	58·10	46·20	0·3753	0·2592	0·4461	0·5610
13	58·30	52·30	0·2965	0·2951	0·5062	0·5642
14	53·23	49·55	0·2690	0·1074	0·2018	0·2168
15	52·56	48·05	0·4815	0·1298	0·2470	0·2701
16	55·16	45·50	0·3850	0·1502	0·2723	0·3301
Total	462·84	411·10	2·9108	1·4683	0·3172	0·3693

result. In Tables III and IV the figures thus obtained are compared with the total values for the eight eggs and eight chicks dealt with in Tables I and II. As in the latter case the eggs used were taken indiscriminately from various farmers, whereas the eggs analysed together were specially selected hatchable eggs obtained from the dealer, we give in another column the total values for the eggs analysed separately after eliminating the abnormally heavy and abnormally light eggs, Nos. 1, 5, 11, and 15.

The percentages of cholesterol in eggs and chicks calculated from the data obtained by analysing a number of eggs or chicks together are nearer the truth than the averages obtained from the analyses of single eggs or chicks, as in the latter case the errors of the various estimations would be

Table III.

	Weight of eggs in grammes.	Weight of unsaponifiable matter in grammes.	Weight of cholesterol in grammes.	Percentage of cholesterol.
6 eggs analysed together...............	359 ·00	2 ·4025	1 ·7578	0 ·4896
8 eggs analysed separately	457 ·55	2 ·9488	1 ·7509	0 ·3827
6 eggs analysed separately; same as above, eliminating 1 and 5	337 ·52	2 ·2199	1 ·3910	0 ·4121

Table IV.

	Weight of eggs in grammes.	Weight of chicks in grammes.	Weight of unsaponifiable matter in grammes.	Weight of cholesterol in grammes.	Percentage referred to weight of egg.	Cholesterol referred to weight of chick.
6 chicks analysed together ...	340 ·2	302	1 ·7805	1 ·5914	0 ·4677	0 ·5270
8 chicks analysed separately	462 ·84	411 ·1	2 ·9108	1 ·4683	0 ·3172	0 ·3693
6 chicks analysed separately; same as above, eliminating 11 and 15	342 ·73	301 ·91	1 ·9878	1 ·1952	0·3487	0 ·3958

accumulated. A comparison of the figures again shows that no gain in cholesterol takes place during the incubation of the chick. Whether the cholesterol of the egg remains unchanged, or whether some loss occurs, cannot be definitely decided.

Conclusions.

In the differentiation of the ovum into the complex aggregates of cells constituting the chick, no formation of cholesterol takes place. This is in accordance with our view that cholesterol is not synthesised in the organism.

This work has been carried out with the help of a grant which was made to us by the Government Grant Committee of the Royal Society, for which we take this opportunity of expressing our thanks.

On the Cross-breeding of Two Races of the Moth Acidalia virgularia.

By Louis B. Prout, F.E.S., and A. Bacot, F.E.S.

(Communicated by Leonard Hill, F.R.S. Received January 8,—Read February 25, 1909.)

A. Introduction.

The general interest which has been aroused of recent years by the various researches which have been undertaken in investigation of the working of Mendel's Law of Heredity, and the adaptability of the Order Lepidoptera to such investigation, have led us not only to reconsider the results of some earlier and undirected experiments in moth-breeding, but also to seek out some peculiarly suitable species in order to take in hand a more exhaustive course of study along the lines most likely to yield further results in elucidation of Mendelism.

Résumé of some Previous Rearing Experiments.

Perhaps a brief reference should be made to our previously recorded attempts at pedigree-breeding.

Lasiocampa quercûs.—Crossings of the various local races were carried out extensively by A. Bacot and J. C. Warburg in 1896–1900, and the results of their work detailed in 'The Entomologist's Record,' vol. 13, pp. 114–116, 237–240, 256–259, 313–317, 338–342. The outstanding feature, as regards a possible bearing on Mendelism, is that two races from the same geographical region, when hybridised, produced progeny that segregated into the two parent forms, whereas when the southern French var. *meridionalis*, Tutt, was crossed with the Scottish var. *callunæ*, Palmer, no such segregation occurred, the larvæ being of an intermediate type.

Forres — Triphæna comes (*Agrotis comes*, Stgr. Cat.).—Some rather unsystematic breeding experiments with the interesting Forres forms of this species were made in 1902–1903 by ourselves and others, and are recorded in 'The Entomologist's Record,' vol. 15, pp. 217–221; vol. 16, pp. 1–5. The progeny from two wild melanic females segregated, that from ♀ A being divisible into 74 typical and 93 melanic, that from ♀ B into 39 typical and 22 melanic. From brood A offspring was obtained, namely, a batch from a single melanic pairing and a batch from melanic "stock"; the former gave 25 typical and 52 melanic, the latter 20 typical* and 48 melanic;

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* "Nine" in 'Ent. Rec.,' vol. 16, p. 3, line 19, is a *laps. cal.* or misprint for "seven."—L. B. P.

the total, therefore, 45 typical and 100 melanic. Thus the segregation was Mendelian in its completeness, but less so in its proportions.

Cluny.—A little later, A. Bacot followed up this experiment with another on the same species, this time with material from Cluny, Aberdeenshire. The results are recorded and discussed in the 'Proceedings of the Entomological Society of London' for 1905, pp. lxvii–lxxi,* and more briefly in 'The Entomologist's Record,' vol. 17, pp. 340–341. In the generation F^1 60 per cent. of non-melanic against 40 per cent. of melanic were reared from melanic ♂ × non-melanic ♀. In F^2 100 per cent. non-melanic appeared from non-melanic parents, the melanic ♂ grandparents showing no influence; on the other hand, from extracted *melanic* pairings of like parentage, F^2 consisted of: in two broods, 30 per cent. non-melanic to 70 per cent. melanic; in another brood, 21 per cent. non-melanic to 79 per cent. melanic. In F^3, so far as tested, both forms bred true, *i.e.*, two pairings of non-melanic × non-melanic produced non-melanic offspring only (6 and 22 specimens respectively), and two pairings of melanic gave melanic only (24 and 12 specimens respectively). The strain was becoming weakly through inbreeding, and here died out. There is some suggestion here that non-melanic is recessive to melanic, though some of the proportions are rather inexact.

Xanthorhoë ferrugata (*Coremia unidentaria*).—A long series of experiments, extending from 1894 to 1898, was undertaken by L. B. Prout with a view to obtaining light on the curious colour-dimorphism of this species, and the results have been published in considerable detail in the 'Transactions of the City of London Entomological and Natural History Society' for 1897, pp. 26–34, and Tutt's 'British Lepidoptera,' vol. 5, pp. 61–64, and summarised in a later memoir, entitled "*Xanthorhoë ferrugata* and the Mendelian Hypothesis" ('Trans. Ent. Soc. Lond.,' 1906, pp. 525–531). These showed, as Mr. L. Doncaster pointed out in an interesting supplementary note ('Proc. Ent. Soc. Lond.,' 1907, pp. xx–xxii), *roughly* Mendelian proportions on the assumption that the black form was recessive to the purple. It seems to us curious, however, in spite of the large percentage of deaths which introduced a factor of indefiniteness, that one pairing of heterozygotes (No. 3 on p. 528 of the paper) should have yielded in F_1 11 specimens showing the recessive coloration as against only 6 showing the dominant—the "expectation" being 4 recessive against 13 dominant, or at best 5 against 12. At any rate, the species shows nearly complete segregation, and will be a valuable one for future Mendelian research.

C. dominula.—We may further mention that Mr. L. W. Newman, of Bexley, a careful and successful breeder of Lepidoptera, has recently observed apparent Mendelian dominance in the typical form of *Callimorpha dominula* over its yellow-hindwinged aberration *rossica,* and of *Abraxas grossulariata* over the aberration *varleyata.* He has very obligingly furnished us with statistics, so far as the experiments have yet gone; and as they are hitherto unpublished, we take this opportunity of putting them on record. Of *C. dominula,* a type ♂ × *rossica* ♀ paired in 1906 produced in 1907 a brood consisting entirely of typical specimens; a pair of these gave in 1908 the following result: 34 typical, 10 ab. *rossica*—there was a great mortality among the larvæ before and during hibernation, fully 60 per cent. dying. Of *Abraxas grossulariata,* a type ♂ × *varleyata* ♀ paired in 1907, produced in June–July, 1908, a brood consisting entirely of typical specimens; pairings from these gave, as a partial second brood, October–November, 1908, 24 of the type (including one aberrant but *not* varleyata) and 7 ab. *varleyata*—the rest of the larvæ now hibernating.

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* On p. 13, line 8, "45 %" is a misprint for "40 %."—L. B. P.

After some consultation we decided upon the small geometrid moth known as *Acidalia* (or *Ptychopoda*) *virgularia,* Hüb., as meeting the essential conditions. There are, be it observed, practical difficulties to be encountered with many species, which have been overlooked by theorisers on the nature of the work that *ought* to be done by Lepidopterists. Thus many moths are exceedingly difficult to pair in confinement; many are extremely averse to inbreeding, so that an inbred strain cannot be continued beyond two or three generations; many are difficult to bring through the winter, or require food-plants which are not always obtainable.

Convenience in Rearing.—*Acidalia virgularia,* on the other hand, will feed, apparently, on almost anything belonging to the vegetable kingdom, leaves of all sorts—whether fresh or withered—sliced carrot, etc., proving equally acceptable to it; it pairs very readily, is continuously-brooded throughout the summer, feeds up rapidly and generally without need of hibernation, and does not deteriorate through continuous inbreeding; moreover, its small size is a practical convenience both for the accommodation of large numbers of larvæ in a small space and for bringing large numbers of the set moths under the eye at the same time. Few, if any, other British species would offer all these advantages to the same degree; and as *Acidalia virgularia* produces in the south of France a race so different-looking from the British that more than one British field naturalist on seeing it has failed to recognise even the species, it is not difficult to trace the influence of the respective parent strains in crossings.

Origin of Stock.—Ova and pupæ of the southern French form were kindly supplied by Mr. H. Powell, F.E.S., from Hyères; wild moths of the London form by Mr. J. E. Gardner, of Clapton, N.E. The former race is distinguished by its white or cream-coloured ground, almost devoid of grey dusting, and scarcely variable except in the intensity of the transverse black lines, which may be strong (the tendency in the particular strain with which we worked), or broken up into dots, or obsolescent; the latter is variable within limits, but always with the pale (not white) ground-colour profusely dusted with dark grey atoms, whether these be uniform throughout or more concentrated in certain areas. The Hyères form is therefore described in our experiments as "light" (L), the London form as "dark" (D). It is necessary to add that more or less intermediate phases of variation occur in some parts of Germany, Italy, etc., so that we have been dealing with *local races* rather than fixed recurrent "aberrations" or with incipient species.

The first cross-pairing was obtained on June 21, 1906, and filial generations I–X (F_1 to F_{10} on the well-known Bateson method) appeared from August, 1906, to November, 1908. Altogether, 5531 specimens have been analysed in preparing these notes, so that they may be regarded as fairly comprehensive as an indication of the behaviour of this particular cross-pairing.

B. STATISTICAL RESULTS.

General Remarks.—Altogether our breeding of *Acidalia virgularia* has been carried out to the tenth filial generation, and 5531 specimens have been subjected to careful analysis, exclusive of a few which have been more indefinitely summarised but which are confirmatory of the general results. We feel that we may, therefore, speak authoritatively on the general course of inheritance in the cross-breeding of these races, and that the imperfection of our statistical analysis is not due to ignorance of the forms with which we are dealing, but to the fact that their hybridisation really gives *no segregation capable of analysis by the human eye.* It is necessary to dwell somewhat on this point. At first sight it might appear a confession of incompetence to have to state—as we do quite frankly—that our figures are only approximations, and that in many cases a re-count (either by another entomologist or even by ourselves) might easily result in a slight modification of them; but when it is understood that there is, in the cross-breeds, every conceivable intergrade, it must be manifest that the distinction between "dark" and "intermediate" on the one hand, and "light" and "intermediate" on the other, becomes purely one of degree, and it is absolutely impossible to draw a perfectly consistent line throughout. Having made a special study of the family *Geometridæ* for nearly twenty years, one of us (L. B. P.) can at least claim to have acquired that eye for slight differences in them that will have safeguarded him against any material error of judgment in the present investigations.

As regards the pure stock, or presumable homozygotes, inbreeding for ten generations, under more or less artificial conditions, has not had the very slightest influence on the pretty Hyères form nor, in the aggregate, on the London form. Excepting a single dark specimen, which was obviously an accidental importation (perhaps on food-plant, as the moth is so common in Mr. Bacot's neighbourhood), upwards of 400 specimens show not the slightest deviation from the clean whitish ground-colour which characterises the Hyères race. The dark form, which varies more in a wild state, naturally showed a greater range, and one or two broods, apparently by some accidental selective agency, became lighter than the normal; but here, again, we can confidently affirm that, among some 400 specimens, none have occurred which could possibly be mistaken for the "light."* It follows, therefore, that the bulk of those which we have classed as "intermediate" can be with certainty explained as blends originating from the hybridisation.

Crosses obtained.—Cross-pairings were obtained in each generation, usually in reciprocal crosses, and in not a few instances in duplicate. In many cases the progeny of the crosses was also carried on down to the generation collateral with that of F_{10} from the original cross. The complete scheme upon which we intended to work may be indicated as follows:—

Pure dark (D).—F_1 to F_{10}.
Pure light (L).—F_1 to F_{10}.
Dark by light (called cross-pairing *A*).—F_1 to F_{10}.
Light by dark (cross-pairing *a*).—F_1 to F_{10}.
Dark by light, ex F_1 (*B*).—F_2 to F_{10}.
Light by dark, ex F_1 (*b*).—F_2 to F_{10}.
Dark by light, ex F_2 (*C*).—F_3 to F_{10}.
Light by dark, ex F_2 (*c*).—F_3 to F_{10}.
Dark by light, ex F_3 (*E*).—F_4 to F_{10}.
Light by dark, ex F_3 (*e*).—F_4 to F_{10}.
Dark by light, ex F_4 (*F*).—F_5 to F_{10}.
Light by dark, ex F_4 (*f*).—F_5 to F_{10}.
Dark by light, ex F_5 (*G*).—F_6 to F_{10}.
Light by dark, ex F_5 (*g*).—F_6 to F_{10}.
Dark by light, ex F_6 (*H*).—F_7 to F_{10}.

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* One curious strain is dealt with separately below, as its actual origin is altogether problematical.

Light by dark, ex F_6 (*h*).—F_7 to F_{10}.
Dark by light, ex F_7 (*I*).—F_8 to F_{10}.
Light by dark, ex F_7 (*i*).—F_8 to F_{10}.
Dark by light, ex F_8 (*J*).—F_9 to F_{10}.
Light by dark, ex F_8 (*j*).—F_9 to F_{10}.
Dark by light, ex F_9 (*K*).—F_{10}.
Light by dark, ex F_9 (*k*).—F_{10}.

Or in tabular form (see p. 139).

The actual hiatus in the carrying out of this scheme will be seen from the details which follow. The number of the dark × light crosses that faded out would suggest some inherent tendency to weakness in this rather than in the reciprocal cross; yet the oldest hybrid of all was a dark × light (*A*) and continued vigorous to the last. None of the hybrid strains was labelled "*D*," this letter being reserved for the pure dark strain.

In addition to these systematic crossings, a few quadroon broods and complex crossings of hybrids were obtained.

Most of the continuations of the broods were from single pairings, but occasionally—as when a number of specimens had emerged simultaneously and we could not be sure that they had not mated unobserved—we bred from stock. The question of the influence of individual parental characteristics as opposed to broader racial ones was not the least interesting in connection with our work.

It is to be remarked that the percentage of deaths in the early stages was generally quite insignificant, and that many of the broods reared to the imago state were so rich in individuals relatively to the fecundity of the species, that our statistics are incontrovertibly much more representative than those obtained from *Xanthorhoë ferrugata*, where the pupal deaths were often enormously numerous.

Since it is impossible to forecast what statistics may ultimately assume unexpected importance, the following record tends to err, perhaps, in the direction of over-completeness. Such deductions as we have been able to draw from the mass of figures will be reserved for the next section of this paper.

Brood *A*.—This was started in duplicate, one of the strains being lost at F_5. The reciprocal cross (*a*) was not obtained, as we had no dark ♀ of assured virginity.

(1) In the generation F_1 there were 62 specimens, all true intermediates with variation inconsiderable. In F_2, 66 specimens, variation considerable, 5 quite dark (perhaps less *brownish* than the pure Clapton race), others

Gen.	D.	L.	D × L. *A.*	L × D. *a.*	D × L. *B.*	L × D. *b.*	D × L. *C.*	L × D. *c.*	D × L. *E.*	L × D. *e.*	D × L. *F.*	L × D. *f.*	D × L. *G.*	L × D. *g.*	D × L. *H.*	L × D. *h.*	D × L. *I.*	L × D. *i.*	D × L. *J.*	L × D. *j.*	D × L. *K.*	L × D. *k.*
1	×	×	×	×																		
2	×	×	×	×	×	×																
3	×	×	×	×	×	×	×	×														
4	×	×	×	×	×	×	×	×	×	×												
5	×	×	×	×	×	×	×	×	×	×	×	×										
6	×	×	×	×	×	×	×	×	×	×	×	×	×	×								
7	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×						
8	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×				
9	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×		
10	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×

approaching this or mottled or banded with dark; none approaching the pure light form. In F_3, 21 specimens, variation slight, follow their actual parents *very closely*—*i.e.* all were intermediates. In F_4, 54 specimens, variation rather considerable in ♀'s, less in ♂'s; 2 ♀ dark, much as in F_2; several quite as light as F_1, none pure light. In F_5, 47 specimens, variation rather considerable, none very light, 7 (4♂, 3♀) dark.

(2) In generation F_1 all were true intermediates, though somewhat variable; a subordinate race-characteristic was perhaps adumbrated, which became more pronounced in a few specimens of each brood from A_2 to A_6, namely, a tendency to darkening in the *outer area* of the wings (a common characteristic of some species of *Acidalia*, such as *A. politata*, etc.). In F_2–F_6 the variability increased, but according to no fixed rule; in F_6 (20 specimens) the range was from almost pure light (very weakly marked) to almost pure dark, with intergrades. Two pairings were obtained in F_6, one of light × light, the other dark × light; the former yielded, as F_7, 47 specimens of remarkable constancy, most of which might be called pure light, three perhaps light-intermediate; the latter yielded, as F_7 (from fine dark ♂ × light ♀), some half-dozen specimens only. F_8, from the former of the two F_7 broods, consisted of 13 pure light and one (♂) intermediate; another F_8, from *stock* (of the latter of the two F_7 broods), also of 14 specimens, differed strikingly from its cousin brood in tone, all being intermediate, and somewhat variable. F_9 was again duplicated, one batch (labelled *A* ix ⊙), from light parents, consisted of 81, the majority light, but only about 30 per cent. pure light; the other batch (*A* ix ✱), also from light parents, gave 6 only, all pure light. F_{10} (ex. *A* ix ⊙), 21 specimens, ranged from light (1 or 2) to true intermediate (2 or 3), the majority light but *very slightly dusted.*

Brood *B.*—Carried only to the fifth generation (strictly speaking, the *fourth* generation; but, as shown in our "scheme," it has been thought better throughout to give uniform numbering to collateral lines, *i.e.*, to regard as F_2 the grandchildren of the original stock even though with strain *B* the crossing of the two races only commenced a generation later, with strain *C* two generations later, and so on). In generation F_2 there were 14 specimens, variation inconsiderable, all intermediate, lines rather weak. In F_3, 34 specimens, variation not great, similar to parent brood, but 3 or 4 distinctly light—beginning to "throw back" towards ♀ grandparent. For F_4 a duplicate pairing was obtained; one brood, 31 specimens, averaged distinctly paler than F_3, several closely approaching the pure light; the other brood, 52 specimens, also varied little, but more closely resembled the parent brood, only 3 or 4 (♀'s) being whitish the rest intermediate. In F_5, from the former of the two F_4 broods, 32 specimens varied little, though not absolutely inappreciably, all light, yet not quite so pure as the original Hyères strain.

Brood *b.*—Obtained in duplicate, one strain carried on to generation F_5, the other to F_{10}.

(1) An interesting strain on account of an apparently hereditary predominance of the female sex, figures therefore given in full. In F_2 (28 ♂, 36 ♀) all were intermediates, though somewhat more variable than most first crosses. F_3 was duplicated; one brood (13 ♂, 17 ♀) showed considerable variation, ranging, in both sexes, from pure dark, through intermediates, to nearly, but not quite, pure light; the other brood (13 ♂, 24 ♀) also varied rather considerably: 3 almost pure dark, a few others approaching these, others intermediate or lightish, 7 with a characteristic facies, almost of the pale, well-lined Hyères form, yet less extreme and less white. F_4, from the former of the F_3 broods, consisted of 14 ♂, 25 ♀ : both sexes quite variable, several ♂'s dark, 1 or 2 ♀'s light (not strongly lined), the ♀'s thus averaging somewhat the lighter. In F_5 (17 ♂, 27 ♀) the range of variation was much as in F_4. Adding the above numbers together, we find that this strain yielded only 85 ♂ against 129 ♀, a proportion of 2 : 3.

(2) This proved on the whole a very stable strain, though generations F_4 to F_6 varied more. F_2 was very uniform, intermediate. F_3 (7 only) similar, may have been a shade darker and a few showed a dark *border*, which became a feature of the strain. F_4 distinctly variable, though not quite reaching either extreme; a dark *central* shade, quite a feature of some, lacking in others. F_5 (19 only) very similar to F_4, but smaller, and perhaps hardly so variable. F_6 (66) strongly variable, particularly in the expression or suppression of the two rows of transverse dots, there being a sudden outcrop of specimens in which they are very pronounced—none such being observable in F_2 to F_5. Where these dots are on a white ground (14 specimens), "pure light" is produced; the rest are intermediate to dark, none very dark. F_7 consisted of 3 only, intermediate, weakly marked, but 2 with the borders darkened. F_8, 23 specimens, a singularly uniform brood, a phase of "intermediate" without strong dusting or lines of dots, the distal margin often darker. F_9 was duplicated, but both the broods (46 and 11 specimens respectively) closely followed F_8, though a few in the larger brood were a little more heavily dusted. F_{10} was almost a failure, only one (intermediate) specimen coming through.

Brood *C.*—Continued to generation F_9. In F_3 there were only 3 poor specimens, apparently intermediate, but no exact analysis possible. F_4, a large batch, moderately variable, range from almost pure light to darkish but hardly dark; about half tend toward the light side. F_5 are similar to F_4, but perhaps less variable, very few pure light; might in the aggregate be termed light-intermediates. F_6, 36 specimens, are pretty variable, about 11 light (only with a stronger central shade than in the pure Hyères strain); 1 or 2 others nearly as light, weaker-marked; the rest intermediate to darkish, nothing extremely dark. The darkest pair available was used for parentage of F_8. F_8 (4 ♂, 16 ♀) are rather large, variable, the average dark, could perhaps be classified as 11 (3 ♂, 8 ♀) dark, 5 (♀) intermediate, 4 (1 ♂, 3 ♀) light, though not quite pure, but there are very gentle gradations; duplicate pairings out of F_7 (labelled *C* viii (2) and *C* viii (3)) vary somewhat less, *C* viii (2) (33 specimens) being intermediates, slightly variable in detail, and *C* viii (3) all being possible London forms (*i.e.*, dark), variable only in detail. F_9 was obtained only from the second of the above (*C* viii (2)); the specimens numbered 35, still intermediates, not unlike the parent brood.

Brood *c*.—Continued to generation F_{10}. F_3 consists of 7 only, intermediates, apparently not variable. F_4 varies from almost extreme light to almost extreme dark, but with intermediates which preclude any possibility of splitting up into darks and lights. F_5 (16 only) is similar, but the preponderating tendency is on the dark side, only one being really light with strong lines of dots. F_6, 29 specimens, is perhaps even more variable, 4 or 5 at least being pure dark, 6 or 7 at least pure light, others nearing both extremes (especially the light side) and a few intermediate. F_7 (from light ♂ × dark ♀) showed quite moderate variation, most being rather uniform, lightish intermediate, 2 or 3 (♂) darker, without being strikingly dark. F_8, 52 specimens, are very variable: about 12 pure dark, about 12 pure light (only with well-expressed central shade), the rest grading through. F_9 was bred in triplicate; brood *c* ix ⊙, 30 specimens, from intermediate parents, are very uniform, all being possible Clapton forms (dark), only 2 or 3 a little paler than would be normal for Clapton; *c* ix ✻, 7 specimens, from lightish parents with distinct dot-lines, follow the parents closely; the remaining brood (stock ?), 56 specimens, shows moderate variability, but mainly intermediate, the few darks and the few lights hardly quite pure. F_{10} was nearly a failure, but 5 specimens ex brood *c* ix ⊙ are all dark, 6 ex *c* ix ✻ all lightish, reproducing their parents' facies.

Brood *E*.—Only continued for two generations. The original pairing, ex generation F_3, was obtained in duplicate.

(1) In generation F_4 55 specimens were reared, all intermediate, the variation not considerable. In F_5, 83 specimens, the variation is considerably greater, ranging from darkish (not extreme) to specimens closely approaching the pure light strain, though slightly less pure, with central shade better indicated.

(2) In generation F_4 58 were reared, all intermediate and remarkably constant. Excepting the slight sexual dimorphism, the variation might be said to be practically *nil*. Progeny not obtained.

Brood *e*.—Continued to generation F_7. F_4 consisted of 77 specimens, all intermediate except, perhaps, one brownish ♀, which resembles some of the lightest London forms; the other 76 exceedingly constant. In F_5, 49 specimens, the variation is much greater, ranging from a few of each sex quite resembling the London forms to a few whitish, though certainly not pure. F_6, 78 specimens, extremely variable, though not definitely segregating; a few very dark, several darkish, one darkened in outer area, numerous intermediate, numerous light or lightish, the black dot-lines then generally (not always) well expressed, some with, some without, the dark central shade, 2 or 3 agreeing fully with the pure light strain. In F_7 only 6 moths were bred, from stock, variable from dark to light.

Brood *F*.—Continued to generation F_8. The original pairing was duplicated.

(1) F_5, 29 specimens, singularly enough, acted differently from *all* the other first crosses, being virtually a pure light brood, and we hoped that, for once, the light ♀ parent had acted as a dominant. Fortunately a large offspring was obtained (from stock) consisting of 150 specimens. These (F_6) are much more variable than F_5, but cannot be split up into light and dark definitely; roughly classified, we made 45 light (perhaps a dozen *pure* light), 95 intermediate, 10 dark (none extremely), but the gradations are so extremely slight that a re-count would be almost sure to modify the figures somewhat. Two pairings were obtained: one brood of F_7 (ex light ♂ × dark ♀) yielded 7 specimens, all more or less intermediate, 3 more dusted than the other 4; the other brood (ex light ⚥, with strong dot-lines), 6 specimens, all rather light, but only one with the lines sharp. F_8, from a pair of the lightest specimens in the former of the last-mentioned broods, again proved numerically inadequate, only 7 coming through; these are rather variable, 6 being on the lighter side (2 or 3 pure, the others grading towards intermediate), the seventh strongly dusted (dark intermediate). Attempts to continue the strain proved unsuccessful.

(2) F_5 here consisted of 47 specimens, the variation not considerable, the general facies being very uniform, but the colour ranging from lightish (not pure) to a lightish intermediate. Offspring was not obtained.

Brood *f*.—Continued to generation F_{10}, though then on the verge of extinction. F_5, not variable, would certainly be classed as true intermediate, though rather on the light side. F_6, 64 specimens, is much more variable, two or three being pure light, several others closely approaching it, many intermediate, and a few rather dark. F_7 (ex light ♂ × dark ♀) consists of 32 specimens, extremely uniform, all intermediates, with fairly distinct dot-lines. F_8, 44 specimens, again vary, yet with no absolutely pure white, and only 3 or 4 very *strongly* dark-dusted, nothing extraordinarily dark. Duplicate pairings were here obtained; from a rather dark ♂ × rather light ♀, sprang, as F_9 (labelled *f* ix ⊙), 28 specimens having a similar range to F_8; from an apparently intermediate pair, the ♀ darkened in outer margin, 33 specimens, rather constant, intermediate, nearly all well dusted on a whitish ground. F_{10}, 2 specimens only, agree with the F_9 brood last mentioned, from which they sprang.

Brood *G*.—This cross was obtained, but not propagated beyond the single generation (F_6). The brood consisted of 69 specimens, slightly more variable than most first crosses, yet in no way startling. Most are quite normal intermediates, 2 or 3 might better be classed as dark, yet not extreme,

Brood *g*.—Continued to generation F_{10}. In F_6, 35 specimens, the variation is inconsiderable, all being intermediate, though such variation as there is is towards the "light" side. F_7 was obtained from a lightish pair, and yielded 4 lightish specimens. F_8, 47 specimens, was again rather constant, a light-intermediate. F_9 was obtained in duplicate; one batch (labelled *g* ix ⊙, parents rather weakly marked) consisting of 18 specimens, intermediate, nearly all weakly marked, the colour ranging from darkish to lightish without extremes; the other batch (*g* ix ✻, from a better-marked pair) considerably variable, 49 specimens, mainly well-lined, about 12 almost the pure Hyères form, 3 or 4 approaching the London form, many intermediate. F_{10}, 47 specimens from the last-named brood, follows it well on the whole, nearly all being well-lined, though there is much variation in tone and many (especially of the darker ones) are rather strongly darkened towards the outer margin.

Brood *H*.—Continued to the second generation of the cross, that is, to F_8. F_7, 56 specimens, is very constant, and very typical of the normal "first cross"—all intermediate. F_8, 49 specimens, is very variable; hardly any are quite pure light, only 1 or 2 pure dark (and not very intense); but there is almost every other variation, in size, strength of markings, general facies, and ground-colour.

Brood *h*.—Continued to generation F_{10}. In F_7, 12 specimens, the variation is very slight, all being lightish intermediate. F_8, 36 specimens, is decidedly variable, the range being from pure light to darkish intermediate, with the usual intergrading. F_9 was duplicated; one batch (from light, well-lined parents) yielded 17 specimens, variable from pure light (though not intensely white) to intermediate, 11 or 12 having the lines rather strong; the other batch yielded 16 specimens, hardly variable, intermediate to light-intermediate, weakly lined for the most part. From the former of these batches sprang, as F_{10}, a brood of 17 specimens, rather constant, with a uniform facies which struck one as recognisable even when they were emerging; all are intermediate in colour, the dusting weak, the lines rather strong.

Brood *I.*—Obtained but not carried on. The single family (F_8) consists of 46 specimens, intermediate, decidedly constant.

Brood *i.*—Continued to generation F_{10}. F_8 consists of 22 specimens, rather constant, normal intermediates. F_9 was obtained in duplicate; from one pair (labelled *i* ⊙) resulted a very variable brood of 32 specimens: 5 or 6 pure light, others near, 7 or 8 pure dark (some quite extreme), others near, and various intergrades; from the other pair (labelled *i* ⟐) another variable brood, of 12 specimens only, mostly lightish-intermediate, 1 almost pure light, though slightly brown tinged, 1 pure dark, 2 darkish. F_{10} was reared from both these broods; that from the former consisted of 44 specimens, intermediate to dark, presumably from some of the darker examples among the parent stock; the latter of 25 specimens, varying in colour from white to intermediate, yet with a most conspicuously definite facies, all being well lined, with the central shade strong and clear cut in addition.

Brood *J.*—F_9, 29 specimens, intermediate to lightish-intermediate, fairly constant. F_{10}, 5 only, certainly variable, though without extremes—altogether too few for generalisations. The reciprocal cross (*j*) was not secured.

Brood *K.*—Obtained in duplicate. Both batches (56 and 38 specimens respectively) normal intermediates, the variation slight. Some undersized specimens look a little pale, but this is because of their weak scaling.

Brood *k.*—40 specimens, variation moderate, from light-intermediate to dark-intermediate.

The quadroons and other irregular crosses have next to be briefly dealt with. In the first filial generation pure light ♂ was crossed with hybrid ♀ (out of the brood described as *A*, number (2) in this paper), and the strain carried on for four generations (F_2 to F_6). It continued "intermediate," with the variation appreciable but not considerable, only in F_6 there were more of the whiter specimens. In generation F_6 this quadroon race was crossed with the hybrid race called *f* in this paper; the variation in the offspring (F_7) was only very moderate, ranging from light to lightish-intermediate.

Also in generation F_6, crosses of $b \times c$, $b \times f$, and $G \times C$ were obtained, but only $b \times f$ was followed up to subsequent generations. All these three were interesting, as in each case both the parents were more or less extreme, the ♂'s light and the ♀'s dark; the offspring of *b* × *c* (16 specimens) varied little, all being intermediate or lightish; that of *G* × *C* (4 specimens only) much more, the single ♂ bred being darkish, the 3 ♀'s light, weakly marked. In this generation (F_7) the specimens of *b* × *f* (34 in number) varied little, the range being from lightish-intermediate to lightish, almost reaching the pure Hyères form; the characteristic dark border of the parent strain *b* (2) entirely disappeared. In F_8, 47 specimens, the variation was considerably greater, ranging from pure light (about 8) to pure dark (2 or 3), the majority intermediate, and the extremes not very intense. F_9 was obtained in triplicate; from a light pair (especially the ♀) sprang a brood of 34 (labelled *bf* ⌺ ix), hardly variable, all light or lightish; from a somewhat intermediate pair a brood of 44 (labelled *bf* ⊙ ix), variable, from lightish (not extreme) to dark—about 8 that might be likened to average London specimens; from stock a batch of 23 (labelled *bf* ⊗ ix), slightly variable, all light or lightish except 1, which is intermediate, brown. In generation F_{10} one brood was raised, simply labelled *bf* x, the note of its *exact* parentage having unfortunately been mislaid; it consists of 32 specimens, nearly pure light and not varying much, a few virtually of the Hyères form, but the larger number with a fairly distinct central shade.

In generation F_8 a pairing was obtained between a ♂ out of brood *H* (intermediate or darkish, weakly-marked) and a ♀ out of brood *c* (intermediate or rather light, the central shade distinct). In generation F_9 a brood of 33 appeared, rather variable, from light-intermediate to dark (not intense), mostly weakly lined, a few strongly freckled. Their progeny (F_{10}, 19 specimens) are also variable, from light (3) to dark (5); 4 are intermediate, fairly well scaled, the rest more or less poorly scaled, weakly-marked.

It remains to notice a strain which must be treated as of uncertain ancestry, and which originated in F_6 and has been carried on to F_{10}. It was believed to have sprung from pure dark ancestry, a number of hibernating larvæ of F_3 in that strain having fed so slowly as to be still in the larval state when their nephew-brood of larvæ (*i.e.* pure dark F_4) arrived, and having been mingled therewith; but in F_6 the behaviour of the strain was so unprecedented that we feel forced to imagine there must have been some accidental importation of hybrid or light material, inexplicable though it is, considering the care that was taken. Of course, it is open to those who so desire to assume that there was here a true mutation, but as the white form has never been known in Britain, and inbreeding has not changed the rest of our pure dark stock, we ourselves cannot regard such a view as even worthy to be provisionally entertained, unless confirmation be forthcoming.

Brood *D* ✻.—This aberrant stock in F_6, which we called *D* ✻ vi, consisted of 41 specimens, 18 of them pure light, 2 nearly pure but browner in ground-colour, a few normal intermediates, and about 12 typical dark. By analogy with the rest of our material this would suggest being a second generation from a hybrid. On account of the riddle of its origin, 5 pairings from this brood were obtained, 2 others attempted proving infertile.

(1) From a light pair sprang, in F_7, a brood of 16 (labelled *D* ✻ vii (2)) all light, about half being quite extreme, the rest slightly more dusted.

(2) From another light pair, F_7 consisted of 47 (labelled *D* ✻ vii (3)), rather variable from pure Hyères form (4 or 5) to intermediates. Their progeny in F_8 (64 in all, from different pairings) varied conspicuously, the *majority* light to intermediate, perhaps only one really *dark*, and that not very extreme. In F_9 (two broods, 67 specimens) the variation was less, only ranging from light to intermediate; all the four actual parents were more or less light. In F_{10} (three broods, 96 specimens) the variation again increased somewhat, but with the lighter forms still in the ascendant and thoroughly dark ones only occurring, and sparingly, in *one* of the three broods—labelled *D* x (1) ⊙, and noteworthy for its darker average tone than its parent brood.

(3) From yet another light pair, F_7 (26 specimens, labelled *D* ✻ vii (4)) bred absolutely true to the extreme parent form (= wild Hyères type). One pairing produced, in F_8, a further brood of 24, all equally pure, unfortunately lost here. Another pairing produced, in F_8, a very *variable* brood of 45—15 pure light, about 15 others lightish to light-intermediate, the rest darker, 1 or 2 practically "dark." From a pairing of rather light, well-marked specimens in the latter of these (the variable) was obtained, in F_9, a brood of 38 varying much less than the parental one, indeed pretty constant light-intermediate, rather well-lined. Their offspring (F_{10}, 42 specimens) would nearly all be classed as "light-intermediate" in some sense, yet wonderfully variable within this limit; whitish weak-marked, similar examples but greyer behind the outer line, moderately light strong-marked, intermediate (2 or 3 strongly-marked, 2 or 3 weaker-marked) are all represented.

(4) From a dark pair, only three specimens were reared in F_7 (labelled *D* ✻ vii (6)). These were darkish intermediate. Fortunately a ♂ and a ♀ emerged together and copulated. The resultant F_8 (49 specimens) varied a good deal, one only being pure light, the rest about half intermediate (a few light-intermediate) and half darkish to dark, but with intergradations. F_9 (78 specimens, 3 broods) was moderately variable, but all should be classed as broadly "intermediates." F_{10} (49 specimens) was variable, ranging from intermediate to *dark*, the average darker than in F_9; perhaps about

20 would be called dark, but there is no clear line of demarcation. The return to a darker type might be called atavistic, but more probably the actual parents—which are not known—happened to be among the darkest ones of F_9.

(5) From a dark ♂ × light ♀, F_7 consisted of 21 specimens (labelled *D* ✻ vii (7)), all normal intermediates, with no appreciable variation. The strain was unfortunately lost.

C. General Conclusions.

From the foregoing mass of detail a few facts emerge with conspicuous clearness, and certain other points are sufficiently suggested to be worth putting forward, at least tentatively.

In the first place, there is most certainly no Mendelian dominance in coloration in the cross of the dark (London) race of *Acidalia virgularia* with the light (Hyères) race. With remarkable persistence, a first cross of the pure races produced a form *intermediate in coloration.* The sole exception, out of two dozen such crossings, is the brood noticed above as *F*, No. (1).

But, in the second place, it is perfectly well known that colour-dominance is not the essential feature of Mendelism. As Mr. Bateson says,[*] "The essential fact which Mendel discovered is the segregation of characters in gametogenesis." Now, as the intermediate form, which was so nearly universal in the first crosses, did not appear in either of the "pure" strains, it may well be taken as the normal manifestation of hybridity in this blend, corresponding to the "blue" Andalusian fowl and other well-known cases; and it is certainly noteworthy that a rough resolution into a wider range of forms proved quite general in the F_2 generation. That proportions did not agree with expectation might be due to defective analysis. For example, the said "hybrid" or "intermediate" might have a wider range of variation than had been discovered by the investigators, who might thus have referred some hybrids to one of the "pure" forms. But a glance at our actual results convinces us that it is not generally too *few* intermediates that we obtained in F_2 but too *many*; and fortunately we know very accurately the limits of the variation of at least one of the pure races (L), so that there seems no chance, on the assumption of gametic purity, of our having classified pure "lights" as "intermediate." It is, however, further noteworthy that some, at least, of the extracted strains (light × light, ex hybrid, viz., *A* (2) in generation F_7,[†] ? *B* in generation F_5 (1), *b* × *f* ⛋ in generations F_9 and ? F_{10},

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[*] 'Progressus Rei Botanicæ,' 1906, p. 368.

[†] But if this was really "pure" whence came the single "intermediate" ♂ in its offspring?

? *D* ✻ (1) in generation F_7, ? *D* ✻ (3) in generations F_7 and one section of F_8;* dark × dark, ex hybrid, viz., *C* (3) in generation F_8, *c* ⊙ in generations F_9 and F_{10}) attained a considerable standard of purity; and also that a few of the extremest (light × dark) pairings among hybrids (such as $b \times c$, $b \times f$, *D* ✻ (5), and ? A (2) in generation F_7) were the most reliable in producing again genuine intermediates. ($G \times C$ was possibly an exception, but the parents here were not so extreme in colour as to render hybridity unthinkable.)

Another fact that can be stated with certainty is that our experiments have revealed no other decisive "reversion to type" than the kind which Mendelism would demand; the intermediates have been quite as stable as Mendelism would expect in hybrid pairings. Whatever be the explanation, it would appear that the hybrid form cannot be "bred out"; except in cases where a selective mating has been employed and the rest of the brood allowed to die out, intermediates have continued to appear through all the generations.

Without desiring to dogmatise, we feel it is necessary to remark that neither of the points last considered—the obtaining of a comparatively uniform type by selective mating and the persistence of intermediates under other circumstances—belongs exclusively to any one theory of heredity, while such occurrences as those noticed in the footnotes on broods A_2 and *D* ✻ (3) are harder to reconcile with Mendelism than with, for example, the Galtonian view. On the whole, the apparently large responsibility of direct parenthood suggests to us the idea of some such principle as is involved in the well-known formula of one-half the characters from the parents, one-fourth from the grandparents, etc.

Over and over again some trifling race-characteristic has interested us in a particular strain, including—besides the tendency for some broods to favour the slightly darker variations and others the lighter—obvious differences in the expression or suppression of the transverse "dot-lines," tendency to develop a dark central shade or a dark marginal area (for instance, brood *b* (2)), and so forth. Any of these would have been well worthy of minute study, either from a Mendelian or a non-Mendelian point of view, had time and opportunity allowed. We suspect, however, that in large measure they also would be found traceable to direct parentage, for it is certain that in some cases cousin-broods differ quite materially in some of these characteristics, and that a reference to their parents shows how closely these are followed; see, for instance, some of the references under the statistics of broods *c*, *g*, *h*, etc. The sex-predominance in brood *b* (1) was another peculiarity which deserved more attention than it received.

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* But how would Mendelism account for the (very variable) *other* section of F_8?

We noticed also that the larvæ were very variable, and it is not impossible that an analysis of their variation might yield some results of value.

As a final impression, we would suggest that our failure to find Mendelian inheritance at work was due mainly to our bringing together two comparatively remote geographical races (as with Messrs. Warburg and Bacot's *Lasiocampa meridionalis* × *callunæ*) and that we, personally, now only expect to find segregation in the case of crosses of two forms occurring together (like the two forms of *Triphæna comes* or those of *Xanthorhoë ferrugata*), where a long course of natural selection has presumably eliminated the intermediates. We pointed out in the introduction that just such intermediates of *Acidalia virgularia* as were produced artificially by crossing our specimens from London and Hyères (localities where they are apparently quite unknown in a wild state) do occur in a state of nature in other parts of its geographical range.

A few pairings which occurred in generation F_{10} produced ova which have been handed to Mr. W. Bateson in the hope that he may be able to follow up our researches in the species. Unfortunately both the pure strains have been lost, but possibly Mr. Bateson will be able to extract them, by selective pairing, from the new hybrids, which we labelled *M* xi and *m* xi.

The Nerves of the Atrio-ventricular Bundle.

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[Plates 4—6.]

Physiologists, in explaining the transmission of the wave of contraction in the heart from atrium to ventricle, have alternately leaned towards either the myogenic or the neurogenic hypothesis. Many of the discussions on this subject have been useless and many of the deductions false, because of the misconception of the anatomical facts. For long it was held that though in cold-blooded vertebrates the experiments of Gaskell had settled the question in favour of the myogenic theory, yet in mammals there existed an interruption between the atrial muscle and the ventricular muscle at the atrio-ventricular groove, and that this was opposed to a general acceptance of the myogenic theory. Later anatomical investigation, however, has definitely shown that there exists in all mammals, between the atria and the ventricles, a pathway of modified muscle fibres along which the contraction wave appears to go. This is the atrio-ventricular (auriculo-ventricular) bundle, or bundle of His. The discovery of this muscular connection gave very decided support to the myogenic hypothesis, and at present it would appear that prevailing opinion favours this theory. It receives additional support in so far that in this bundle only a few nerve fibres have as yet been recognised by one or two observers; some assert that nerves are not present, or, if so, are too few in number to be of any moment. More definite statements have been made by Tawara and Retzer. Tawara (1) found that "in the heart of the calf the atrio-ventricular bundle is accompanied by a very considerable nerve bundle, which runs with the muscle bundle, and in the left ventricular septum nerve cells are present 1·2 cm. below the aortic valve." In the atrio-ventricular bundle of the sheep only a few nerve bundles were seen, but in the dog, cat, and in man he could find none, though he expressly states that these cannot be excluded, since fine nerve fibres accompany the bundle. Retzer (2) has pointed out that the first ganglion cells that appear in the embryonic heart lie in the atrial septum, immediately above the beginning of the conductive system; further, that the Purkinje fibres are surrounded by a plexus of non-medullated nerves.

Such is the present position of our knowledge of the nerve constituents of the atrio-ventricular bundle. Although a vast amount of work has been done on the nerves of the heart generally, yet, so far, but little attention has been directed to this particular and definite strand. In this preliminary paper I have limited the report to the muscle band as it passes from the atrium to the ventricle, and shall not discuss the nerve constituents in the connections of the atrio-ventricular bundle with the ordinary muscle of the atrium or of the ventricle; that is, I limited the investigation to that part which extends from a point in the bundle towards the coronary sinus over the bifurcation into right and left branches and down these into the right and left ventricles. These parts are represented in figs. 1 and 2 (Plate 4).

Historical.—The first specific demonstration of the presence of a muscular connection between the atrium and ventricle was given by Gaskell in 1883. Previous to that time many writers had declared that such a connection existed, but their statements were indefinite. For instance, Paladino (3) is referred to by Bardeleben as having found that "die Vorhofsmuskulatur endet nicht an den Annuli fibro-cartilaginosi, sondern geht grossentheils in die Ventrikelwand und die Papillarmuskeln weiter." It was Gaskell (4) who first definitely showed that in the tortoise the contraction wave spreads from the sinus over the auricle to the ventricle by means of the muscular connection which exists between the three parts of the heart at the sino-auricular and auriculo-ventricular grooves. He found that at the sino-auricular junction the fibres of the sinus form a circular muscle-ring from which the fibres of the auricle take origin. From this origin the fibres of the auricle, after ramifying in all directions, approach and get attached to the upper and middle part of the auriculo-ventricular groove, forming a ring of muscle fibres from which in turn the fibres of the ventricle take origin. By experimentally sectioning between the two auricles, and by removal of the visceral pericardium, he was able to show that the "ventricle contracts in due sequence with the auricle, because a wave of contraction passes along the auricular muscle and induces a ventricular contraction when it reaches the auriculo-ventricular groove." The integrity of the whole muscle at the auriculo-ventricular groove is unnecessary for this sequence, for there exists a definite track along which the wave of contraction passes. Histologically he found that the muscle fibres present in the auriculo-ventricular muscle-ring differed from the muscle cells of the auricle and ventricle both in the size of the nucleus and the character of their striation.

In spite of Gaskell's work, the hypothesis generally accepted was that the contraction wave cannot be myogenic because in mammals there occurred a distinct break between the muscle of the atria and the muscle of the ventricles. It was held that the atrial fibres and the ventricular fibres belonged to independent systems, and were separated by a considerable amount of connective tissue at the atrio-ventricular junction. In 1893, ten years after Gaskell's work appeared, Stanley Kent (5) showed that muscular connection did exist in the mammalian heart. He found in young rats that there was at birth a well-defined continuity of atrial and ventricular muscles. As age advanced there took place a considerable development of connective tissue in the atrio-ventricular groove; nevertheless, in adults there still persisted well-marked bands of muscle tissue between the two chambers over a considerable area of the atrio-ventricular groove, and in particular at the junction of the atrio-ventricular septum. This muscular connection varied in amount in different animals, yet could be demonstrated in all; but the exact location of these bands he did not definitely determine.

In the same year there appeared the work of Wilhelm His, junior (6), "Die Tätigkeit des embryonalen Herzens und deren Bedeutung für die Lehre von der Herzbewegung beim Erwachsenen." Here for the first time was given the definite location of the atrio-ventricular bundle. His demonstrated that in the earliest stages of development of the mammalian heart there exists a continuous muscle union between the heart segments, and that the primitive contraction goes on without the presence of ganglion cells. Further, it was erroneous to suppose that this primitive continuity of muscles was completely interrupted in the adult by connective tissue at the atrio-ventricular groove; that while a break did occur it was by no means complete, for at a particular place in the atrio-ventricular septum muscular union persists. This union is brought about by a strand of muscle fibres which springs from the posterior wall of the right atrium, passes forward to the atrio-ventricular groove lying in the upper part of the ventricular septum, soon forking into right and left branches. The presence of this bundle of His has been confirmed by many subsequent writers.

The most important work on the atrio-ventricular bundle is that of Tawara (1), "Das Reizleitungssystem des Säugetierherzens." This Japanese investigator, working in Aschoff's laboratory in Marburg, published, in 1906, a careful and exhaustive monograph on the macroscopic and microscopic appearances of the bundle in a series of mammals. In it he demonstrated the connections of the fibres of the bundle with the ordinary cardiac muscle and the relation of the Purkinje fibres throughout the heart to the atrio-ventricular fasciculus. His findings (7) may be summarised as follows:—

(1) In man and all the animals examined, the Purkinje fibres or their equivalents form the outspreading of a muscular system which unites the atrial muscle to the ventricular muscle. This muscular system constitutes the atrio-ventricular bundle.

(2) This muscular system has a uniform arrangement in all mammals, though slight individual differences appear. It runs from the atrial wall through the atrio-ventricular fibrous septum to the point where it spreads out in the ventricular wall, at first as a bundle enclosed in connective tissue, then spreading out into tree-like end branches. The closed strand never enters into relation with the ventricular muscle, but the end branches fuse with the usual ventricular muscle.

(3) This uniting system is early developed in the embryo. From this time on, leaving growth out of consideration, it remains unchanged during life. It is *not* affected by hypertrophic and atrophic processes in the heart in the same way as the ordinary cardiac muscle.

(4) The topographical, histological, and biological peculiarities of this system are opposed to the suggestion that its function is that of a heart pump, like the ordinary cardiac muscle. Moreover, the physiological experiments of Gaskell, Engelmann, Herring, and others suggest that in this system can be found a conducting path for the co-ordination of the heart muscle.

Macroscopic Description of the Bundle.—To dissect out rapidly the atrio-ventricular bundle, I have found it best first to identify the pale pink muscle fibres of the right and left branches. These are more or less subendocardial. The left is readily observed at a varying distance beneath the junction of the posterior semilunar (non-coronary) with the right semilunar valve. The right branch, less readily found in some mammals because covered by a thin layer of ventricular muscle, can be found lying near the line which joins the moderator band to a point under the medial (septal) cusp, adjacent to its junction with the anterior (infundibular) cusp of the tricuspid valve. From either of these points the entire atrio-ventricular band can be dissected out.

As seen in the calf, where it is very large, the bundle originates in the posterior wall of the right auricle in the region of the sinus coronarius (Plate 4, figs. 1 and 2). At this point there is a mass of pink fibres—not red like the ordinary cardiac fibres, and less pale than the main continuation of the band. It is cone-shaped in appearance, with an ill-defined base merging into the auricular muscle and a well-marked apex passing into the narrow band of white tissue which goes forward into the atrio-ventricular septum in a groove on the under surface of the cardiac cartilage. Reaching the upper part of the interventricular septum, it divides into a right and a left branch. The right branch passes downward on the septal wall of the right ventricle more or less superficial towards the septal attachment of the moderator band. Here it begins to separate out and send branches into the septum. If a transverse section be made of the moderator band, Purkinje fibres are seen in small bundles isolated by more or less definite connective tissue sheaths. Through the moderator band it reaches the lateral wall of the ventricle and the papillary muscles. The left branch appears under the endocardium of the septal wall of the left ventricle about 1·5 cm. below the junction of the posterior with the right semilunar valve. Immediately under the aortic opening it is covered with cardiac muscle. On reaching the surface of the heart it spreads out and sends branches downwards and outwards to the septal and lateral walls of the ventricle.

In mammals generally, the course of the atrio-ventricular bundle agrees in the main with this, but some slight differences are observed. Thus in the sheep the band is smaller and less distinct. The right septal branch is covered by cardiac muscle from the atrio-ventricular septum to the moderate band; the left branch appears under the endocardium 1·2 cm. beneath the junction of the right and posterior aortic valves. In the pig there is a close resemblance to this. It is interesting here to note how in all mammals the bundle is in close apposition to the insertion of the aorta, behind its posterior valve.

Microscopically, as Tawara pointed out, one must also distinguish an atrial and ventricular part. The atrial part begins as a complicated network of branching fibres smaller than the atrial muscle cells with a nucleus lying in undifferentiated protoplasm and with fibrillæ less well developed and more irregular than in the ordinary cardiac cell. Within this network lie connective tissue with fat, blood-vessels, and nerves. From it emerges a series of more or less parallel muscle fibres similar to those of the network surrounded by a well-marked connective tissue sheath. The appearance of these cells and the amount of connective tissue between them readily distinguish them from the ordinary cardiac muscle.

The ventricular section begins immediately where the atrio-ventricular bundle breaks through the fibrous septum of the atrio-ventricular groove. It consists of an irregular network of muscle cells surrounded by connective tissue. Histologically it has no similarity with the atrial portion of the band nor with the ordinary cardiac muscle cell. One, two or more of the cells of the atrial strand pass into a much larger cell of irregular size and shape which possesses many features in common with a Purkinje cell. As the ventricular strand passes down the septum, these initial cells gradually pass into typical Purkinje fibres which constitute the muscle cell component of the two arms and their outspreading branches.

Technique.—As a first step, it is necessary to be able rapidly to cut out the bundle either after staining or in the fresh condition. This can be done after a series of preliminary dissections. For the purpose of this research I limited my observations to the main bundle surrounded by its connective tissue sheath, including the part directed to the coronary sinus and the two arms running into the septal walls of the right and left ventricles (figs. 1 and 2). My results have been obtained by the methylene blue "vital" method. Occasionally the Cajal method or gold impregnation was used, but with results less satisfactory, chiefly, I believe, because the methylene blue gave such definite results that it was not felt necessary in this preliminary report to ascertain experimentally the modifications which appeared necessary to apply either of these methods, especially the Cajal, to the heart muscle.

The strength of the solution injected was :—

Methylene blue, $\frac{1}{2}$-per-cent. solution	10 c.c.
Salt solution, 0·9-per-cent. solution	90 „

The coronary arteries were injected with the solution either directly or indirectly from the aorta. When the heart was well injected the bundle was rapidly dissected out, placed on a slide and examined in the usual way; or the bundle was cut out from the fresh heart, partly immersed in a methylene blue solution slightly weaker than the above, and kept in a hot chamber at a temperature of 37° or 38° C. The full description of the technique in use has been so often presented in previous papers, Wilson (8), that it seems unnecessary to repeat it here. Fixation was always done in 8-per-cent. ammonium molybdate and sections cut in paraffin.

The limitations of the methylene blue technique are well known. This dye, though neurotropic, is not monotropic. A difficulty encountered in the atrioventricular bundle is the affinity of the methylene blue for the elastic fibres—a tissue sufficiently abundant in the bundle to give trouble at first. Recognising this possible source of error, one readily gets accustomed to distinguish between the relatively coarse wavy fibres of the elastic tissue and the fine varicose fibres of the nervous strands branching and anastomosing irregularly.

The nerve fibres are in the main non-myelinated. A few medullated fibres are to be seen, especially in the calf: these appear usually as isolated fibres and do not enter as a rule into the strands of fibres which pass directly through the bundle.

The staining of all the nerve elements in the bundle in one and the same preparation is unusual. Thus it is not common to get in the same preparation the finer network together with the ganglion cells and their processes. If one gets good ganglion cells with processes, the finer varicose fibres distributed through the muscle are usually poorly stained. This agrees with the observations which I have satisfied myself with time and again, that the dye will at one time select motor endings in preference to sensory or vasomotor; at another time, under the same external conditions, the sensory or the vasomotor are the better, and may be the only ones stained well. This appears to me to be due to the particular chemical state of the nerve ending at the moment the dye reaches it.

The animals used in the investigation have been the calf, sheep, and pig, and to a less extent the dog. Tissue was also obtained from the human heart. I am not prepared at this time to report on the results of the investigation on human material or in the dog, but so far as these have gone I have no reason to believe that they will not bear out the results reported in this paper on the three first-mentioned animals.

The examination was confined to the part of the bundle extending from near the coronary sinus through the fibrous septum and down both arms. Though this constitutes but a part of the entire bundle, it forms an important section; it is a well-defined structure and includes the path across the atrio-ventricular septum. To avoid confusion, it is referred to in this paper as the atrio-ventricular bundle.

Even a very superficial examination convinces one of the important part taken in its composition by nerve elements. Nerve cells are scattered in profusion along its course, and nerve fibres pass in strands along with its muscular elements or intricately interlace around its cells. There is no part of it, from the coronary sinus to the end of its right or left arm, bereft of groups of ganglion cells or devoid of nerve fibres. The neurologist might well refuse to recognise in it a muscle bundle; to him it might become conspicuously a nerve pathway of very intricate structure.

In the bundle the nerve elements divide themselves for descriptive purposes into three groups:

I. Ganglion cells.
II. Nerve fibres and plexuses.
III. Nerves directly associated with the blood-vessels.

I. The ganglion cells are naturally first described, for they are the most conspicuous nerve structures present, both from their abundance and their size. They are found usually in groups of varying number; some of these in the calf have as many as 16 nerve cells, but more may easily be present. Individual cells, scattered either in the course of the nerve strands or isolated in the fibrous tissue around the bundle, are frequently seen.

Nerve cells are abundant in the atrio-ventricular bundle of all the animals studied, but I have examined them chiefly in the calf, where, from their large size and from the facility with which they stain, they are especially suitable for investigation. All three varieties of cells are found (see Plate 5) unipolar (fig. 3), bipolar (fig. 4), and multipolar (fig. 5). Ganglionic groups are scattered in the connective tissue not only around the muscle band but also in the interstices between the muscle fibres (fig. 4). They are not especially located in the subendocardial area; when the bundle reaches the surface of the heart, they are seen not only between its tissue and the endocardium, but are also equally conspicuous within the bundle and in the fibrous tissue on the side remote from the endocardium.

They can be seen all the way from the coronary sinus to the distribution in the right and left walls of the ventricular septum. In the particular part examined they appear to be most abundant near the point of bifurcation and in the course of the right and left ventricular divisions. Their abundance may be roughly estimated by saying that in a series of sections of one of these arms, especially the left, cut 50 microns thick, it is no unusual thing to find in a section three or four ganglionic masses containing from five to nine nerve cells. Were one roughly to indicate any one area more than another where they are conspicuous, one might select the upper border of the bundle just before its division into right and left limbs. Here there is a large group, easily seen in the calf and sheep, lying in the fibrous tissue outside the bundle whose processes pass into the muscular pathway.

The processes of these nerve cells can often be traced for a long distance gradually dividing in their course (fig. 3). Many of them ultimately become varicose fibres, and some at least go into the nerve plexus around the muscle cells. But the mode of termination of the majority, from the distance they traverse and from the failure to stain referred to above, I have so far been unable to determine. Fig. 6 shows a large nerve cell in some respects akin to Dogiel's type I. It has one long process and several smaller ones, which latter project only a short distance from the cell-like sharp prickles. The long process can be traced for a very considerable distance; it frequently divides and ultimately ends as very fine varicose fibrils which enter into the plexus around the muscle bundle. Fig. 5 shows the interlacing of the processes of several nerve cells in one pericellular plexus. Here a multipolar cell, $G._1$, gives off a branch, one of whose rami enters into the pericellular nest of cell $G._2$, into which also enter twigs from two other more distant nerve cells by fibres C and D.

II. In the atrio-ventricular bundle the nerves present themselves

(*a*) as strands of fibres;
(*b*) as plexuses of fibrils.

(*a*) The nerve strands have a general course along the length of the bundle. In preparations of the entire bundle they are seen to break into the fibrous tissue around it at various parts of its course, but chiefly prior to or near the point of division into the right and left arms. Thus several strands appear towards the coronary side of the division, corresponding to the position of the ganglionic group referred to above. The question of the source of the fibres lies outside the field of the present investigation, but their course indicates an origin near the insertion of the aorta and the lower part of the atrial septum. Sections of the bundle bring out more clearly the relation of these strands to the ganglion cells. Throughout their course nerve cells are scattered individually or in groups and the processes of these cells enter into the nerve strand (fig. 4). The fibres of these strands are not in close apposition. This is especially observed in the left septal part (fig. 4). They are found in the connective tissue both around the bundle and between the muscle cells. They have irregular connection with each other. Often one can see a strand send off a single fibre or a group of three or four fibres which pass across the intervening muscle cells to an adjacent strand and then continue their course along this. Occasionally a fibre may be observed to turn backwards; however, the general tendency is for the strands to pass downwards in the direction of the atrio-ventricular bundle. The fibres are as a rule non-medullated, most of them with varicosities. These varicosities occur at less frequent intervals than in group (*b*). The medullated nerves seen have been chiefly in the cow. It is with fibres of this group that at first the elastic tissue is apt to be confounded, but enough has been said to show how they may be easily differentiated.

It is difficult to tell what becomes of these strands. Many of them pass through that part of the bundle I have examined. Some, however, may be seen to break up into very fine varicose fibrils which enter into plexus (*b*) (fig. 4, A.).

(*b*) The nerve plexuses are composed of very fine fibrils with varicosities at frequent intervals. They can be seen with the 8-mm. objective and 4 ocular, but require a higher power for distinct observation. The fine branches lie in close apposition to the muscle cells and have absolutely no resemblance to elastic tissue. In well-stained preparations they are so dense and intricate from frequent branchings and anastomoses that it is impossible to trace individual fibres for any distance. I have chiefly studied these plexuses in the pig and sheep. Here they form a continuous network which can be traced for long distances over a series of sections and appear to extend the whole length of the atrio-ventricular bundle. Their general characters can be well seen in figs. 7 and 8. As will be noted, it is not a case of a nerve breaking up and surrounding individual muscle cells, but of a complicated network lying around both single cells and groups of cells.

The source of the plexuses is not easy to determine. The difficulty is due partly to the length of the varicose fibrils before they enter intimately into the plexus, and partly to the difficulty of staining at the same time sufficiently well ganglion cells and muscle plexuses. Occasionally, however, a non-medullated fibre can be seen to pass out from the strand and break up into fine twigs, which ultimately become very fine varicose fibrils and enter into direct relation with the muscle plexus. (See Plate 6.)

III. Nerves related directly to the blood-vessels of the atrio-ventricular bundle. These have no special significance in this locality and differ in no way from nerves found in arteries elsewhere, so well described by Dogiel and others. Two distinct varieties present themselves:—

(1) A vasomotor plexus of fine non-medullated varicose fibrils (fig. 9). These are most abundant in the large vessels and gradually get fewer as the smaller arterioles are reached. At the point where a vessel divides or a branch is given off, the plexus becomes more dense; it is as if the fibres became concentrated at the point of division. Then the plexus divides or sends offshoots along the arterial ramus, the amount sent off varying with the size of the branch. The main plexus runs in the adventitia, but a part of it passes into the tunica media to form anastomosing branches directly related to the muscle fibres. These do not appear to me to form definite endings; the knob-like endings on the muscle cell sometimes described appear to be artefacts due to a stoppage of the dye. This appears to be confirmed by relatively few appearing in well-stained preparations and their abundance in badly-stained tissue.

(2) Distinct from these are the so-called sensory endings. These are definite end organs situated in the fibrous coat around the vessel, and differ from the above described anastomosing plexus formations. They are so called because of their resemblance to sensory endings elsewhere. A nerve fibre thicker and less varicose than the ordinary vasomotor nerve and at times faintly medullated is seen to break up in the tunica adventitia into a more or less complex arborisation which is non-capsulated. In the smaller arterioles they are very simple (fig. 9, S.), in the larger arteries of the bundle they become extremely complex (fig. 10).

Conclusions.

I. Anatomically the atrio-ventricular bundle contains not only a special form of muscle fibre distinct from the ordinary muscle of the atrium or the ventricle, but, as I have shown, is an important and intricate nerve pathway in which we find:—

(1) Numerous ganglion cells—monopolar, bipolar, and multipolar—whose processes may pass—

(*a*) To adjacent ganglion cells in the bundle;
(*b*) To the muscle fibres in the bundle;
(*c*) Through the muscle bundle so far as it was examined.

(2) Abundant nerve fibres running through it in strands, the processes of which may end (*a*) in ganglion cells in the bundle; (*b*) in the muscle plexus, or may pass through the part examined.

(3) An intricate plexus of varicose fibrils around and in close relation to the muscle fibres of the bundle.

(4) An abundant vascular supply with well-marked vasomotor nerves and sensory endings.

II. Physiologically it has been shown that the atrio-ventricular band constitutes the pathway which assures the communication of the atrio-ventricular rhythm. When the bundle is sectioned or crushed the ventricles cease momentarily to beat, though they soon regain pulsation, but with a rhythm much more slow than that of the atrium. Pathological anatomy supports this view; the allorythmia or Stokes-Adams disease can be explained satisfactorily by lesions involving this pathway. As a result of these physiological experiments and from these pathological conditions, it has been asserted that the contraction wave must be myogenic. To such a deduction my anatomical findings are opposed. They demonstrate that in these experiments and pathological conditions an important nerve pathway is equally involved with the muscle bundle. Considering the neurogenic as opposed to the myogenic hypothesis from the anatomical standpoint, one must acknowledge that the very complex nerve constituents of the bundle indicate an important nerve pathway and are very suggestive of an intricate nerve mechanism.

Can the atrio-ventricular bundle be regarded as a neuro-muscular spindle? It has recently been stated that "the conclusion derived from the study of the development and cytological structure of the conductive system is that it is a neuro-muscular apparatus akin to the neuro-muscular spindle of voluntary muscle" (Retzer (9)). Similar suggestions have been made by others, and readily present themselves as a possible explanation of the position and structure of the atrio-ventricular bundle. But have we anatomical data on which to base such a conclusion? To answer this it is necessary to consider the structure of the neuro-muscular spindle.

Were one to accept Golgi's definition, made in 1880, that it is "a bundle of incompletely developed muscle fibres surrounded by a special sheath," one might be tempted to acquiesce in the above view, at any rate provisionally. But the work since then, especially of Ruffini (11) and Sherrington (12), has so widened our knowledge of the neuro-muscular spindle that Golgi's definition is now inadequate, and it is with the complex nerve ending which they describe that the atrio-ventricular bundle must be compared.

The essential anatomical points in the structure of the neuro-muscular spindle may be summed up as follows:—

(1) The fibres which go to form the muscle bundle (Weissmann's bundle) in the neuro-muscular spindle have a diameter less than that of the ordinary muscle fibre. The fibres are directly in apposition, and no connective tissue lies between them, though connective tissue lies around Weissmann's bundle and constitutes the axial sheath of Sherrington. The striation of the fibre is usually only marked in the marginal area, and so on transverse section it looks like a Purkinje fibre. Regarding the two ends of Weissmann's bundle, one, the wider end, is muscular; the other tendinous where the axial fibres of the bundle are attached to the fibrous tissue of the capsule or to the tendon of the muscle.

(2) Around it lies a lymphatic space.

(3) There is a distinct capsule of concentric superposed lamellæ of connective tissue. At the tendinous end of the muscle bundle it is thin and adheres to the tendinous portion of the spindle; at the muscular end it is thin and may be absent.

(4) In the vast majority of cases it is fusiform in shape.

Contrast this with the anatomical description given of the atrio-ventricular bundle in this paper, and it will be seen that, apart from the muscle cells being similar to Purkinje fibres, they have nothing in common. This lack of agreement is further emphasised when we compare the nerve constituents of each:—

(5) To the distribution and termination of the nerves in the neuro-muscular spindle, with its three distinct kinds of endings described by Ruffini, there is nothing comparable in the atrio-ventricular bundle.

(6) Ganglion cells are not present in the neuro-muscular spindle, whereas they are a marked feature in the atrio-ventricular bundle.

From the above, one must conclude that, whatever the physiological significance of this bundle may be, it has anatomically nothing in common with the neuro-muscular spindle.

In conclusion, I wish to express my great indebtedness to Dr. Mott for allowing me to carry out a considerable part of this investigation at the Pathological Laboratory at Claybury, and for his kindness in assisting me to procure a large part of the material which has been used.

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EXPLANATION OF PLATES.

A.V.B. = atrio-ventricular bundle.
R.B. = right branch of A.V.B.
L.B. = left branch of A.V.B.
B. = bifurcation.
C.S. = coronary sinus.
M. = muscle.
G. = ganglion cell.

PLATE 4.

FIG. 1.—Dissection of right atrium and ventricle of calf to show A.V.B. The band is seen passing from under atrial muscle near C.S. to B., then continued under medial cusp over right ventricular septum at R.B. towards the insertion of moderator band (M.B.).

V.C.I. = inferior vena cava.
A. = auricle.
M.C. = medial cusp.
A.C. = anterior cusp.

FIG. 2.—A.V.B. of calf looking down from above. The great portion of atrium has been removed to show bifurcation in the upper part of ventricular septum.

R.V.S. = right ventricular septum.
L.V.S. = left ventricular septum.

PLATE 5.

FIG. 3.—Monopolar ganglion cell; one of six lying among muscle fibres of A.V.B. on septal wall of calf. Zeiss comp. oc. 4, obj. 8 mm.

FIG. 4.—Group of ganglion cells lying among nerve fibres in A.V.B. on left septal wall of calf.

A. = nerve fibre breaking up near muscle fibre and entering into muscle plexus (not distinguishable with this low power). Zeiss oc. 6, obj. AA.

FIG. 5.—Ganglion group in A.V.B. of calf, showing pericellular plexus around ganglion cell ($G._2$) containing fibres from three nerve cells.

$G._1$ = nerve cell which sends off process (dendrite) B, which branches repeatedly within ganglionic group. One of these branches breaks up into a network which embraces the adjacent ganglion cell $G._2$.

C. = nerve fibre from an adjacent ganglion—not shown—which breaks up into two branches: from these, twigs pass into plexus around $G._2$, others into plexus around $G._1$.

D. = nerve fibre from nerve cell in same group as C, which breaks into plexus around $G._2$.

E. = varicose fibril clinging to A—neuraxis of $G._1$.

FIG. 6.—Nerve cell in A.V.B. calf, containing a well-marked nucleus with halo. The cell has several small processes which project, like sharp prickles. It has one long process, A, which divides frequently; its ultimate branches become varicose and lie in close proximity to muscle fibres of A.V.B. Zeiss comp. oc. 4, obj. 2 mm.

PLATE 6.

FIG. 7.—Plexus around and adjacent to muscle fibres of A.V.B. of pig, immediately to coronary side of its division into right and left septal branches. Zeiss oc. 4, obj. 8 mm.

FIG. 8.—Plexus around muscle fibres of left septal branch of A.V.B. of pig. Zeiss comp. oc. 4, obj. 2 mm.

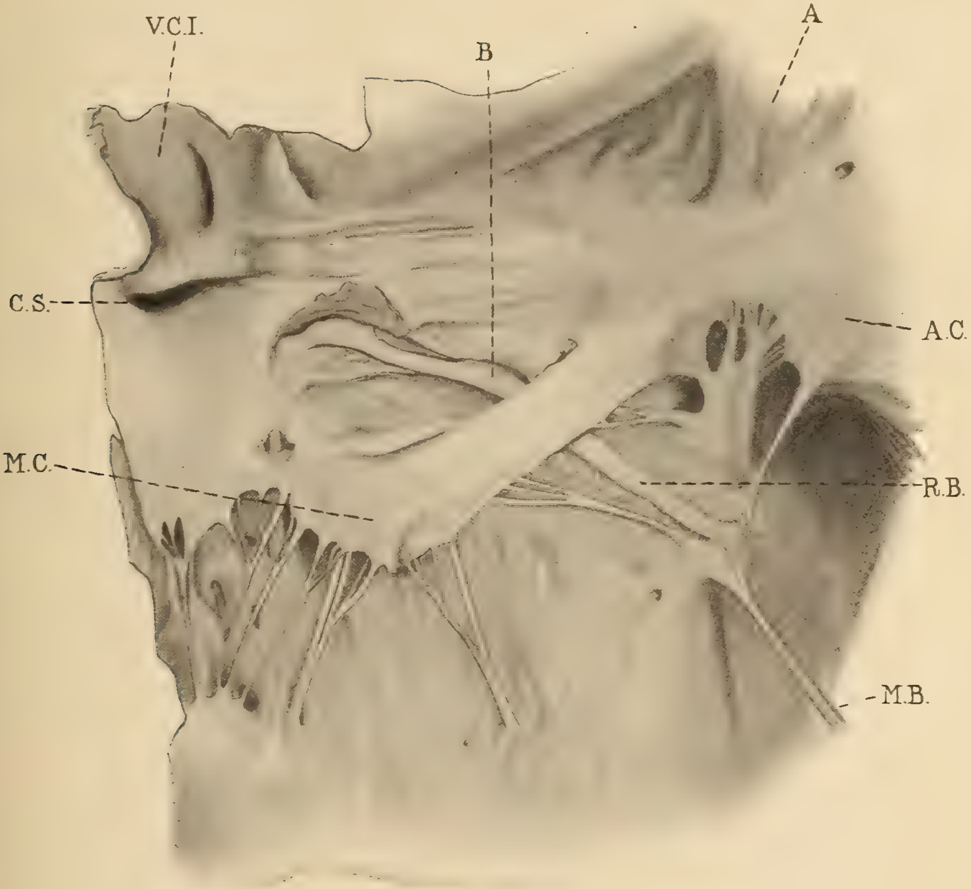
FIG. 9.—Vasomotor fibres in small artery in A.V.B. of pig.

S.N. = sensory nerve, S. = sensory ending, V.M.P. = plexus of fibres at bifurcation of artery.

FIG. 10.—Sensory ending in wall of artery in A.V.B. of cow.

A. = nerve which breaks up into complex ending.

FIG. 1.


V.C.I.
B
A
C.S.
A.C.
M.C.
R.B.
M.B.

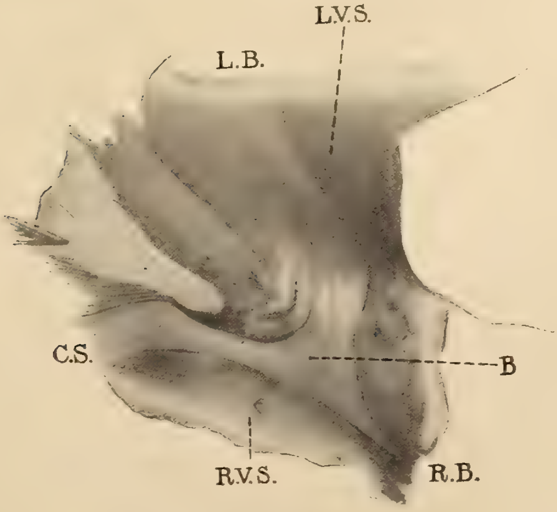

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FIG. 2.

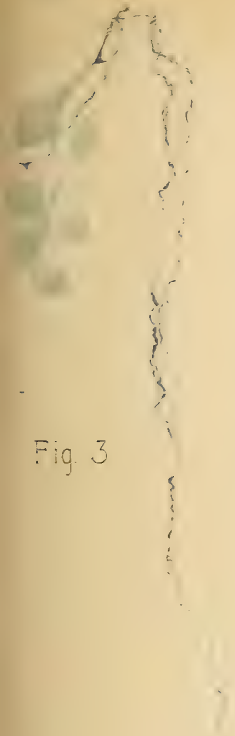
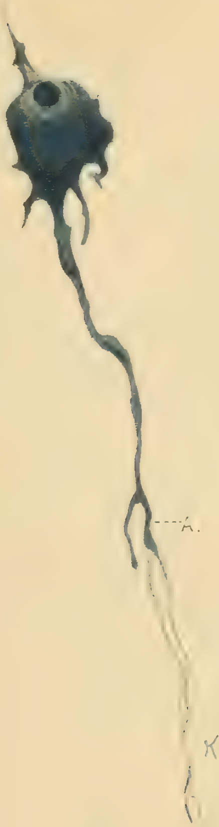


Fig. 3



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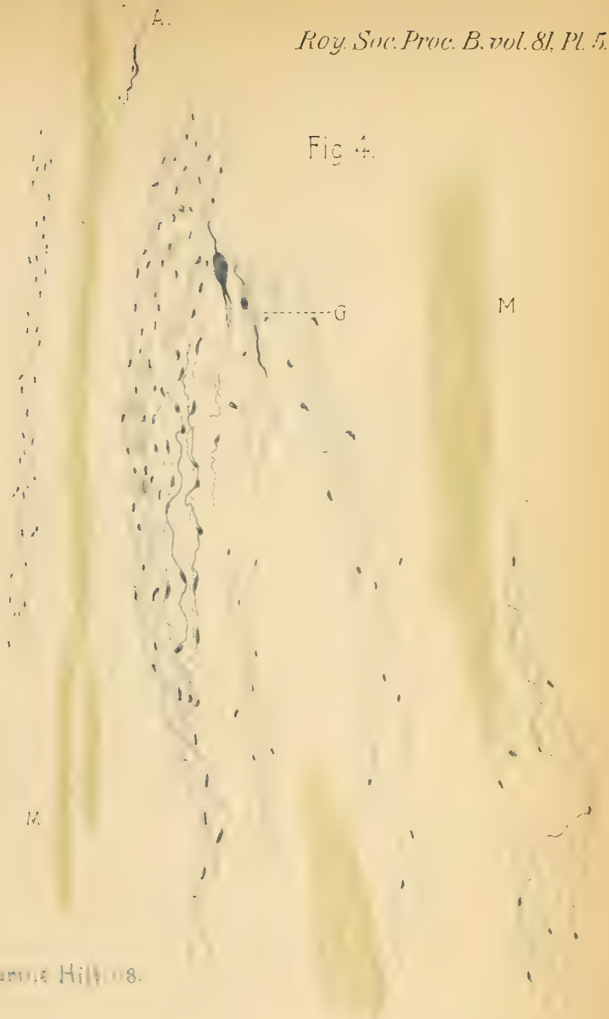

Fig 4.

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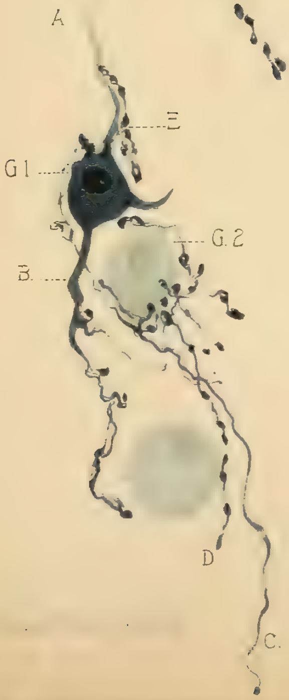

Fig. 5.

Fig. 7

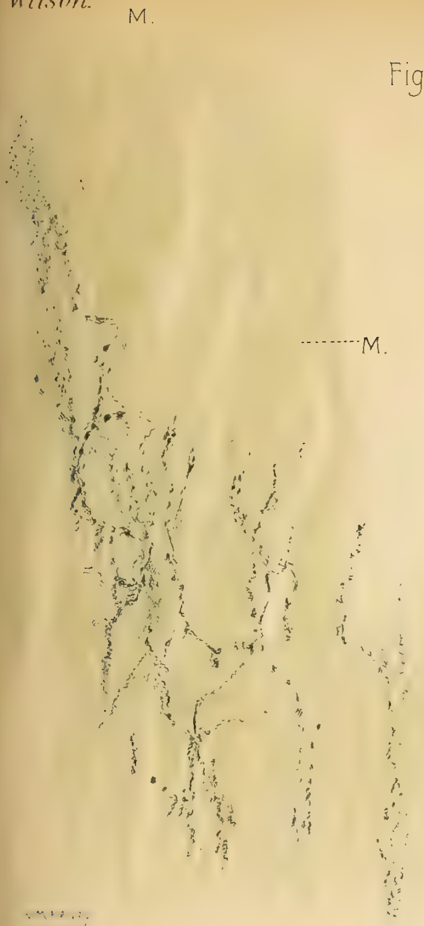

Fig. 8.

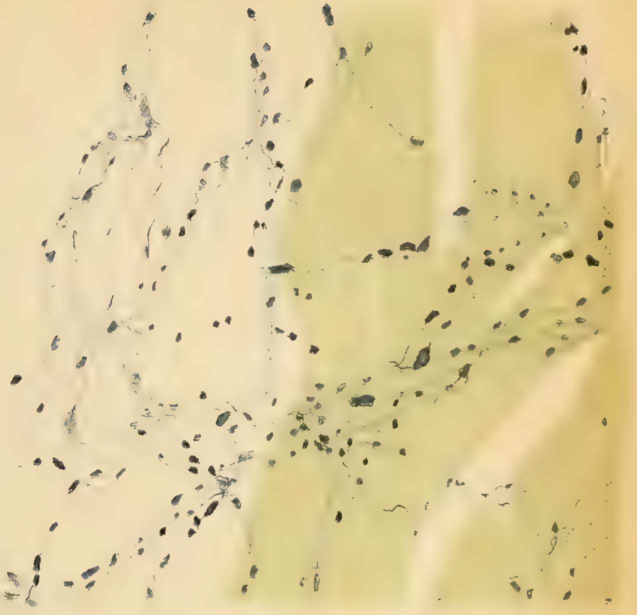

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Fig. 9

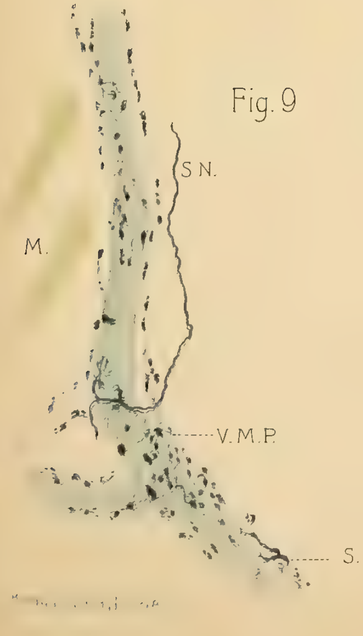
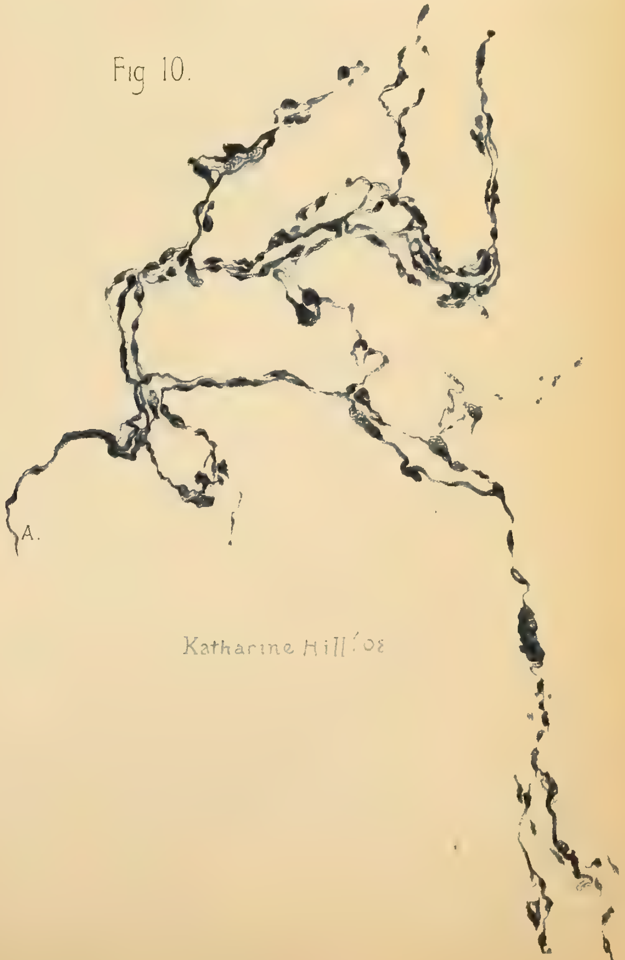


Fig 10.



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The British Freshwater Phytoplankton, with Special Reference to the Desmid-plankton and the Distribution of British Desmids.

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I. INTRODUCTION.

Not until much work had been done at the phytoplankton of the freshwaters of Western Europe were investigations of a similar nature begun in the British lakes and rivers, and it is during the last ten years that almost all our knowledge of this branch of freshwater biology has been acquired.

We have ourselves conducted nearly all the British investigations, and we now think the work has progressed sufficiently to enable us to summarise the results, and to institute comparisons between the British phytoplankton and that of continental Europe and other regions.

Since 1900 we have collected plankton from a large number of lakes in the west of Scotland, from some of the lowland Scottish lochs, from practically all the lakes of the English Lake District and most of those in North Wales, from nearly all the lakes of the west and south-west of Ireland, from Lough Neagh and Lough Beg, from Malham Tarn in West Yorkshire, and from the Rivers Ouse, Lochay, and Bann. In the collection of material for these investigations we have been greatly assisted by four grants from the Government Grant Committee of the Royal Society and two from the Fauna and Flora Committee of the Royal Irish Academy.

From a biological standpoint the British lakes are of great interest, and since the publication of the first reports on their plankton, the diversity of the Algal constituents has been a revelation to the freshwater biologist. In the number and diversity of the species constituting this phytoplankton the lakes of the western British areas stand ahead of any other lakes which have so far been investigated.

The numerous Scottish lakes are almost all of a montane character. They are situated in the vicinity of high mountains and in a northern latitude, and many of the higher plants occurring in these localities in abundance, at almost sea-level, are typical montane species.*

The lakes of the four principal areas examined, the North-west Scottish, the West and South-west Irish, the Welsh, and the English lakes, are situated in the most mountainous parts of the British Islands. As these areas contain the great majority of the British lakes, and are all geographically in the west or north-west, they can be spoken of as the *western and north-western lake-areas.* The mountains amongst which they are situated are composed almost entirely of old formations, and the lake-basins are for the most part drainage areas on outcrops of Older Palæozoic or of Precambrian rocks, often with associated intrusive Igneous masses.

Malham Tarn, West Yorkshire, and Lough Neagh are somewhat isolated lakes, and their phytoplankton is considered separately.

Wherever possible, boats were used for the collection of the plankton, and for one part of the investigation of Lough Neagh, the Lough Foyle and River Bann Fisheries Company kindly lent a steam launch. The smallest tow-net had a mouth-diameter of 6 inches and a length of 15 inches, the largest was 12 inches diameter and about 36 inches in length. The nets were usually towed after the boat at a speed of about 1½ to 2 miles per hour, but on Lough Neagh the large nets were used up to a speed of 4 miles per hour. They were generally kept in the water from 20 to 30 minutes.

We have come to the conclusion that *the best fixing agent is 2 or 3 per cent. formalin, but it should always be replaced before the material is examined.*

Neither picric acid nor chromic acid should be used in plankton-work, as it is almost impossible to wash the material free from these reagents by ordinary methods, and *all the gelatinous colonies are broken up.* Alcohol is also to be avoided, as it causes too great a shrinkage and distortion of the more delicate plankton species.

During the past 18 years we have been acquiring a very extensive knowledge of the distribution of British freshwater Algæ, having made many thousands of collections in all parts of the British Islands. As the lake-areas are the richest parts of the country for freshwater Algæ, these districts have been worked very extensively, and as we did not begin plankton investigations until 1900, we have a much more complete knowledge of the general Algæ-flora of these areas—such as is found in the bogs, lake-margins, streams, on the wet rocks of the glens, etc.—than of the phytoplankton. In consequence of this previous detailed acquaintance with the Algæ of the lake-areas, we are enabled with a considerable degree of accuracy to state which species should be considered as *true constituents of the plankton* and which should not. The other constituents are *casual or adventitious,* and their sojourn in the plankton is both temporary and accidental.

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* Occurring in abundance around these lakes are:—*Festuca ovina*, var. *vivipara*, *Andreæa Rothii*, *A. petrophila*, *Bryum alpinum*, various species of *Rhacomitrium*, *Anthelia julacea*, *Platysma triste*, *Cladonia cervicornis*, *Stereocaulon coralloides*, *Sphærophoron coralloides*, *Lecidea geographica*, etc. The whole district is almost treeless.

The work has been entirely of a qualitative character. We have had neither the time nor the necessary funds to conduct quantitative investigations such as those carried out by Apstein,* Zacharias,† Lemmermann,‡ Volk,§ etc., in Germany, by Wesenberg-Lund|| in Denmark, by Huitfeldt-Kaas¶ in Norway, and by others in Switzerland and the United States.**

We have also met with many difficulties in attempting to acquire a knowledge of the periodicity of the plankton of the British lake-areas. Neither academic duties nor funds have permitted the necessary number of visits to these areas which would be required in the course of a year in order to obtain an adequate idea of the periodicity of the plankton. At the very least, monthly visits would be imperative for reliable results to be obtained; and to collect material from a number of lakes, even in one district, would require several days, not to mention the difficulty, and in some cases the impossibility, of obtaining boats in the winter months. It is likewise no easy matter to find suitable men on the spot of sufficient intelligence to carry out detailed instructions at regular monthly intervals throughout the year.††

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* Apstein, 'Das Süsswasserplankton, Methode und Resultate der quantitativ. Untersuchung.,' Kiel und Leipzig, 1896.

† Zacharias in 'Forschungsber. Biol. Stat. Plön,' vol. 3, 1895, etc.

‡ Lemmermann, "Das Plankton der Weser bei Bremen," 'Archiv für Hydrobiologie und Planktonkunde,' Bd. 2, 1907.

§ Volk, "Hamburgische Elbe-Untersuchung., I u. VIII," 'Mitteilungen aus dem Naturhist. Mus. Hamburg,' Bd. 19, 1903; Bd. 23, 1906.

|| Wesenberg-Lund, 'Studier over de Danske Söers Plankton, Kjöbenhavn,' 1904.

¶ Huitfeldt-Kaas, 'Planktonundersögelser i Norske Vande,' Christiania, 1906.

** C. Dwight Marsh, in 'Wisconsin Geol. and Nat. Hist. Survey Bull.,' No. XII, 1903; Kofoid, in 'Bull. Illinois State Laboratory of Nat. Hist.,' vol. 8, 1908.

†† We are at present receiving regular periodic collections, with some necessary data, from several of the Scottish lochs and from some of the lakes of the English Lake District. We have been able to arrange for these collections by means of a further grant from the Royal Society. Periodic collections have also been made for a period of two years from one of the large pools of the Midlands. The details of all these collections will be published shortly.

II. SCOTTISH LAKES.

Extensive collections of phytoplankton were made in various parts of Scotland, but especially in the west and north-west, in August, 1901, May and August, 1902, April, July, August, and September, 1903, and August, 1907. The areas comprised Perth, Inverness, Ross, Sutherland, and the Outer Hebrides. We have also examined a number of collections made by Mr. James Murray of the Scottish Lake Survey (Pullar Trust). In all, we have examined phytoplankton from 38 of the most important of the Scottish lochs. The results of these investigations have already been published,* the previous work being represented by a short report by Borge on some phytoplankton from the Island of Mull.† Mr. James Murray has also examined the plankton of a very large number of the Scottish lochs, and has at different times commented upon the occurrence and distribution of the phytoplankton.‡ Further remarks upon the Scottish phytoplankton have been made by Wesenberg-Lund in comparing it with the plankton of the Danish lakes,§ and still more recently a paper has appeared by Bachmann|| comparing the results of Scottish material with plankton from the Swiss lakes.

The Scottish phytoplankton is largely Chlorophyceous, and is conspicuous for the large number and great variety of its Desmids, among which the following are perhaps the most noteworthy: *Xanthidium subhastiferum, Staurastrum anatinum, St. Ophiura,* and *St. jaculiferum.* In some of the lochs, *Mesotænium macrococcum* occurred as a normal constituent of the plankton. This is a most interesting adaptation of a colonial wet-rock species to a limnetic life, with an accompanying reduction in size of the colonies and their assumption of a spherical form. A similar adaptation of the mucous colonies of *Cosmocladium saxonicum* to a limnetic existence is also found in both the Scottish and the Irish lakes, but much less frequently.

In the smaller and more elevated lochs, *Microspora amœna* is a characteristic

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* W. and G. S. West, in 'Linn. Soc. Journ. Bot.,' vol. 35, Nov., 1903; in 'Roy. Soc. Edin. Trans.,' vol. 41, part 3, 1905.

† Borge, 'Algol. Notis. 4, Süssw.-Plankton aus Insel Mull,' Botaniska Notiser, 1897.

‡ *Vide* James Murray, in 'Roy. Physical Soc. Edin. Proc.,' vol. 16, June, 1905; and in various reports, under the direction of Sir John Murray, on the Bathymetrical Survey of the Freshwater Lochs of Scotland in the 'Geograph. Journ.,' 1900—1908.

§ Wesenberg-Lund, in 'Roy. Soc. Edin. Proc.,' vol. 25, part 6, 1905; also in appendix to a subsequent paper, *ibid.*, part 12, 1906.

|| Bachmann, in 'Archiv für Hydrobiol. u. Planktonkunde,' vol. 3, 1907. It should be mentioned, however, that some of Bachmann's records are open to serious doubt. His identifications do not appear to be strictly accurate. We might ask, among many other questions, "What is *Cosmarium lunaria*?" He also copies Tanner-Fulleman's records, some of which appear to be equally doubtful.

constituent of the plankton, and sterile filaments of slender species of *Spirogyra, Zygnema,* and *Mougeotia* occur in abundance. The more slender species of *Mougeotia* are generally the most abundant of these filamentous Algæ, and in some instances they exhibit a coiling of the filaments such as is known to occur in certain of the plankton-forms of *Melosira.* (Consult fig. 1.)

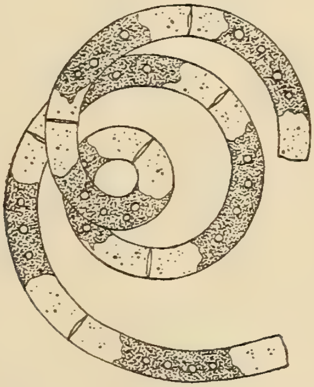

Fig. 1.—A Coiled Filament of one of the Sterile Species of *Mougeotia* from the Scottish Plankton. × 300.

The Protococcoideæ are not very abundant in the deeper Scottish lochs most of them having a decided preference for shallower and warmer water. The most frequent are *Botryococcus Braunii, Sphærocystis Schroeteri, Glœocystis gigas,* and *Ankistrodesmus falcatus.*

Diatoms are conspicuous, largely due to the occurrence in quantity of a few species. They occur throughout the spring and summer in large numbers in the deeper lochs, as the temperature of the water never becomes very high. Many of them are adventitious constituents washed into the plankton from the bogs and shores, but some have become established as true plankton-species. There are about 18 well-established species in the plankton, but only the three species of *Rhizosolenia* are of exclusive limnetic habit, all the remainder occurring in other situations.

The Myxophyceæ (Blue-green Algæ) are very poorly represented in the deeper lochs, and are by no means abundant in the smaller, shallower lakes. The phenomenon of "water-bloom" is very rarely met with, and discoloration of the water, even of the smaller lakes, does not occur either very often or with any great regularity as the result of the accumulation of large quantities of Blue-green Algæ.

The only periodic collections which have so far been reported upon were those made in Loch Ness and examined by Bachmann,* and our own observations relative to the periodicity of the Scottish phytoplankton are amply confirmed by Bachmann's brief report.

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* Bachmann, *loc. cit.*, 1907, pp. 85—88.

Desmids are the dominating constituents in the late summer and early autumn, and there is a preponderance of Diatoms in the colder months, but compared with the seasonal variations of the phytoplankton in some lakes, these differences are not very conspicuous.

Specimens of *Dinobryon* occur in quantity in the spring and summer, and the Peridinieæ are represented by 11 species, although in the larger lakes they are by no means numerous.

Of a total number of 354 species observed in the phytoplankton, 49·4 per cent. are Desmidiaceæ, 17·7 per cent. are Bacillarieæ, and 8·7 per cent. are Myxophyceæ.

III. Lakes of the Orkneys and Shetlands.

We have examined material from one freshwater loch in the Orkneys and from six in the Shetlands. The collections were made in August, 1903, and the results published in 1905.* Mr. James Murray has collected material from 34 lochs of the Orkneys and Shetlands, and has briefly noted a few species of the phytoplankton.†

Although containing a considerable number of species, the phytoplankton was not very rich. Most of the lochs were shallow, and contained quantities of *Asterionella formosa. Pediastrum Boryanum* and *Scenedesmus quadricauda* were both fairly common. Colonies of a *Crucigenia,* described by Wille from the Norwegian plankton as *C. irregularis,*‡ but most probably only irregularly developed forms of *C. rectangularis,* occurred in several lochs of the Shetlands.

Desmids were frequent, and in some cases numerous, but the species were mostly those of shallow lowland lakes. The characteristic western British types were absent, although further investigations in the Shetlands would no doubt bring some of them to light.

Peridinieæ were abundant, as is so frequently the case in shallow lakes, and the ubiquitous *Ceratium hirundinella* was much more generally abundant than in the larger Scottish lakes.

Out of 52 species of Algæ described by Börgesen and Ostenfeld§ as occurring in the plankton of the Faeroese lakes, 28 were observed in the plankton of the Orkneys and Shetlands.

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* W. and G. S. West, in 'Bot. Soc. Edin. Trans.,' Nov., 1904 [1905], pp. 5—10.

† James Murray, in 'Roy. Soc. Edin. Proc.,' June, 1905, pp. 55, 56.

‡ Wille, in 'Nyt Magazin for Naturvidenskb.,' Bd. 38, Heft 1, 1900, p. 10, t. 1, f. 15. Consult also the remarks of G. S. West, 'Treatise Brit. Freshw. Alg.,' 1904, p. 217; and Ostenfeld in 'Hedwigia,' vol. 46, 1907, p. 383.

§ F. Börgesen and C. H. Ostenfeld, "Phytoplankton of Lakes in the Faeroes," 'Botany of the Faeroes,' Copenhagen, 1902.

Of a total of 178 species observed in the phytoplankton, 47·4 per cent. were Desmidiaceæ, 20·9 per cent. were Bacillarieæ, and 9·6 per cent. were Myxophyceæ.

IV. THE IRISH LAKES (WEST AND SOUTH-WEST).

In 1906 we published an account of the plankton of some of the more important lakes of the West and South-west of Ireland,* from collections which we made in May, August, and September, 1904.

In the summer the phytoplankton is greatly in excess of the zooplankton, and the Entomostraca are only dominant in the early spring months. As in the Scottish lakes, the phytoplankton of the lakes of the west and south-west of Ireland is to a large extent Chlorophyceous, but the Bacillarieæ, the Peridinieæ and to some extent the Myxophyceæ, are also conspicuous. The latter are much more noticeable than in the Scottish plankton, especially species of *Anabæna, Oscillatoria, Gomphosphæria, Cœlosphærium,* and *Chroococcus.*

The Desmids are numerous, and include many of the characteristic western types. There is a great abundance of *Spondylosium pulchrum,* var. *planum, Staurastrum anatinum, St. jaculiferum, St. Arctiscon, St. pseudopelagicum,* and *St. paradoxum,* var. *longipes,* but a curious absence of *St. Ophiura.* The latter is a feature of a large proportion of the Scottish phytoplankton, but we have not yet observed it in the Irish lakes. In the lakes examined, the Desmid-flora of the plankton was not quite so rich as that of some of the Scottish lakes, but we think that an investigation of many of the smaller lakes of Galway and Mayo would bring to light a phytoplankton not at all inferior to that of the western Scottish lochs.

Diatoms are very abundant, and form a relatively large part of the Irish phytoplankton. Centric Diatoms are more numerous than in the Scottish plankton, and are represented chiefly by species of *Melosira* and *Cyclotella. Tabellaria, Asterionella,* and the narrow forms of *Synedra* are very conspicuous in the Irish lakes.

The Peridinieæ are generally abundant, and are represented by 10 species. Much the most interesting of these is *Peridinium limbatum* (fig. 2), a characteristic horned species which occurred in some of the small lakes of Galway. It has only been found elsewhere in the United States.

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* W. and G. S. West, "A Comparative Study of the Plankton of some Irish Lakes,' 'Roy. Irish Acad. Trans.,' vol. 33, sect. B, part 2, 1906.

Of a total of 246 species observed in the phytoplankton, 41·7 per cent. were Desmidiaceæ, 19 per cent. Bacillarieæ, and 13·3 per cent. Myxophyceæ.

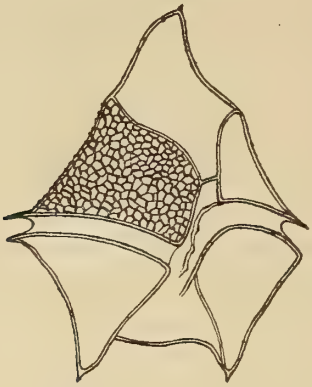

Fig. 2.—*Peridinium limbatum* (Stokes), Lemm., from the Plankton of a small Lake between Clifden and Roundstone, Galway. × 500. The reticulated surface markings of the cell-wall are only indicated on one of the plates.

V. Lough Neagh.

The first contribution to the plankton of the Irish lakes was an account of the plankton-Algæ of Lough Neagh and Lough Beg,* the material having been collected in May, 1900, and July, 1901. This material has since been subjected to a further examination. The lake is so isolated, and, moreover, so differently situated from the other Irish lakes examined, that we have kept the records in a separate column in the tabulated list of British phytoplankton. It is also of interest as being the largest lake in the British Islands; but although covering a large area it is very shallow, its average depth being only 45 feet, and the deepest sounding (made in the north-west corner), is only 96 feet.

The phytoplankton consists largely of Diatoms, Peridinieæ, and Chlorophyceæ. Of the latter, a few of the Desmids characteristic of shallow lakes are abundant, and *Staurastrum paradoxum*, var. *longipes*, occurs in prodigious quantity. *St. pelagicum* was first described from this lake, and has since been found in the lakes of Germany and Iceland. The Protococcoideæ are very well represented, species of *Cœlastrum*, *Pediastrum*, and *Oocystis* being especially noticeable. Of the Diatoms, *Tabellaria fenestrata*, var. *asterionelloides*, is the most conspicuous, but *Coscinodiscus lacustris*, *Cymatopleura elliptica*, and species of *Surirella* are also abundant. The Myxophyceæ are well represented by species of *Anabæna* and *Oscillatoria*, the narrow limnetic species of *Lyngbya*, species of *Cœlosphærium*, *Gomphosphærium*, and *Aphanothece*; and *Chroococcus limneticus* is abundant. Three species of *Dinobryon* are fairly common, but we have no evidence to show whether they ever become dominant or not.

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* W. and G. S. West, "A Contrib. to the Freshw. Alg. of the North of Ireland," 'Roy. Irish Acad. Trans.,' vol. 32, sect. B, part 1, 1902.

On the whole, the phytoplankton is a combination of that which occurs in shallow lakes with that of large pools and ponds. Out of a total of 128 species observed in it, 17·1 per cent. were Desmidiaceæ, 25·7 per cent. other Chlorophyceæ, 32 per cent. Bacillarieæ, and 16·4 per cent. Myxophyceæ.

VI. THE WELSH LAKE-AREA.

Plankton collections were made from 19 of the Welsh lakes, mostly those of Carnarvonshire, during June, 1905, August and September, 1906, and April, 1908.*

The phytoplankton of the spring and summer is essentially Chlorophyceous, and as in the case of the Scottish lakes, is especially noteworthy for the abundance of its Desmids. There is little bulk even in the summer plankton, and in general it has practically no effect on the colour of the water. *The Desmid-flora in certain of these lakes is equal to that found in the richest lakes of the north-west of Scotland, and in one case*—the Capel Curig Lakes—*is superior to that known from any other lake in the world which has been biologically investigated.*

Of a total of 162 species which we have observed in the phytoplankton of the Welsh lakes no fewer than 101 are species of Desmids.

The Protococcoideæ are relatively few, both in number of species and individuals, *Ankistrodesmus falcatus* and *Botryococcus Braunii* being the most frequent.

Diatoms are not conspicuous, and there are fewer species in the Welsh plankton than in either the Scottish or Irish lakes.

Apart from the spasmodic occurrence in fair quantity of one or two species of *Anabæna*, and the moderate abundance of *Oscillatoria Agardhii*, the blue-green element is distinctly scarce in the Welsh plankton.

Species of *Dinobryon* and various Peridinieæ occur abundantly in the Welsh lakes. *Ceratium cornutum* is more abundant than in any other part of the British Islands.

There is no great development of peat in this Welsh lake-area, and the water of the lakes is for the most part clear and limpid. The drainage is mostly down steep mountain sides, with occasional bogs and boggy pools.

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* The details of these collections are being published separately.

The Capel Curig lakes merit special mention. The summer plankton is almost a pure Desmid-plankton, and consists largely of those rare and handsome species which are almost exclusively confined to the west-coast districts of the British Islands. The following species occur in great abundance :—*Micrasterias radiata*, *Staurastrum anatinum*, *St. aversum*, *St. Arctiscon*, *St. Cerastes*, *St. longispinum*, and a stout variety of *St. Ophiura*. It is interesting to note that *St. anatinum* is present in prodigious abundance, as it was from the littoral region of this lake, near the outlet, that it was originally described. *Micrasterias radiata* exists in myriads in the plankton of this lake, occurring in a profusion unknown in any other of its British localities.

Mixed with the Desmids are numbers of *Ceratium cornutum*, and a very few individuals of *Tabellaria fenestrata* and *T. flocculosa*.

Of a total of 162 species observed in the Welsh phytoplankton, 62·4 per cent. were Desmidiaceæ, 11·1 per cent. Bacillarieæ, and 7·4 per cent. Myxophyceæ.

VII. THE ENGLISH LAKE-AREA.

Plankton collections were made from 18 of the English lakes in June, 1903, and September, 1906 ; and since then periodic collections have been commenced in Windermere, Ennerdale Water, and Wastwater.*

As in the Welsh lakes, the phytoplankton of the spring and summer is essentially Chlorophyceous, and contains numerous Desmids, but although most of the typical British plankton-Desmids occur, they are not represented by so many species as in the Scottish or Welsh lakes. The most frequent are the spiny species of *Staurastrum*, *St. lunatum*, var. *planctonicum*, *St. Arctiscon*, *Xanthidium antilopæum*, *Cosmarium subtumidum*, var. *Klebsii*, and *Spondylosium pulchrum*, var. *planum*. The presence of *Staurastrum Ophiura* in the plankton of Easdale Tarn is particularly interesting, as this Desmid is not known to occur in any of the bogs of the English lake-area.

The Protococcoideæ are somewhat scarce, *Glœocystis gigas* and *Sphærocystis Schroeteri* being the only generally distributed species, and these only in small quantity.

The Bacillarieæ and Myxophyceæ are represented by relatively few species, but in some of the lakes Diatoms are dominant constituents of the plankton. Particularly is this the case in Ullswater, in which *Asterionella formosa* was the dominant constituent of both the May and September plankton.

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* As no work has previously been done at the plankton of the English lakes, only the list of species is given for comparison with those of the other British lake-areas. The details of the investigations are reserved for special publication.

Species of *Dinobryon* are common, especially in the early summer plankton. In the May and June plankton of Crummock Water and Derwent Water *Dinobryon cylindricum*, var. *divergens*, completely dominated all other constituents.

Comparison of precisely similar plankton-samples from the various lakes of the English Lake District shows clearly that the proximity of habitations has a distinct effect on the relative bulk of the plankton. *Those lakes which are contaminated by the presence of numerous dwellings and villages along or near the shores possess a relatively greater bulk of plankton than those free from contamination.* The explanation of this fact is most probably the increased amount of nitrates in the water of the contaminated lakes.

Out of 188 species observed in the phytoplankton, 51 per cent. were Desmidiaceæ, 21 per cent. were Bacillarieæ, and 9·5 per cent. Myxophyceæ.

VIII. MALHAM TARN, WEST YORKSHIRE.

This lake is the largest natural sheet of water in Yorkshire and covers an area of 153 acres. It is situated on a limestone plateau at an altitude of 1250 feet, and there is an extensive peat-bog at its northern extremity.

The material was collected by boat on July 23, 1904.

Out of a total of 20 species observed in the phytoplankton nine were Desmids. *Sphærocystis Schroeteri* was very abundant and *Volvox aureus* rather common. Only one Diatom was observed, and four Blue-green Algæ. *Ceratium hirundinella* was very common.

The following is a complete list of the species observed:—*Mougeotia* sp. (sterile), *Gonatozygon monotænium*, *Cosmarium Botrytis* and var. *depressum*, *C. depressum*, *Staurastrum Avicula*, var. *subarcuatum*, *St. brevispinum*, *St. furcigerum*, *St. Manfeldtii*, *St. paradoxum*, *St. teliferum*, *Volvox aureus*, *Pediastrum Boryanum*, *Sphærocystis Schroeteri*, *Surirella biseriata*, *Oscillatoria Agardhii*, *Microcystis æruginosa*, *Merismopedia elegans*, *Chroococcus limneticus*, *Ceratium hirundinella*, and *Peridinium* sp.

IX. THE BRITISH RIVER-PLANKTON.

The first account of British river-plankton (*potamoplankton*) was the comparison of that found in the Upper River Bann with that of Lough Neagh,* and since then Fritsch† has published an account of the phytoplankton of the Rivers Thames, Trent, and Cam. We have also examined the plankton of the Rivers Ouse, Avon, and Cam in England, and the River Lochay in Scotland.

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* W. and G. S. West, in 'Roy. Irish Acad. Trans.,' vol. 32, sect. B, part 1, 1902.

† Fritsch, in 'Ann. Bot.,' vol. 16, Sept., 1902; *ibid.*, vol. 17, Sept., 1903; *ibid.*, vol. 19, Jan., 1905.

It would appear that some phytoplankton occurs all the year round in the British rivers, due mostly to the absence of severe winters, and that in the winter months the living constituents are mostly Diatoms. In fact, *Diatoms dominate throughout the entire year*, the most important genera being *Asterionella, Synedra, Melosira, Surirella,* and *Fragilaria.* Fritsch* summarises the phytoplankton of the Thames in the course of a year as follows :—

Mixed Plankton (with *Asterionella*-phase) → *Melosira* → *Synedra* → Mixed Plankton.

We find *Melosira varians* conspicuous in the spring-plankton of British rivers and persisting in quantity through the summer until the late autumn. *Synedra Acus* is one of the dominant summer species in the British rivers, although it is a spring or an autumn form in the Oder, the Danube, and the Illinois River. *Surirella biseriata* and *S. robusta,* var. *splendida,* are often conspicuous in the summer-plankton, the former being the more abundant, which is never the case in the lake-plankton.

Certain Protococcoideæ occur in the late summer, but never in great quantity. Species of *Pediastrum, Scenedesmus,* and a few other genera are the most frequent, and occasionally odd specimens of a *Cosmarium* or a *Closterium* may occur.

Flagellates and some of the Volvocaceæ occur mostly in the spring months and generally attain their greatest abundance before the maximum summer temperatures. *Endorina elegans* is most irregular in its occurrence, and is often found in quantity in midsummer or even in early autumn. We have never found the Volvocaceæ so numerous in river-plankton as they often become in the plankton of lakes and pools. *Pandorina morum* is the most frequent both in the rivers and small pools.

The backwaters of the rivers are largely the breeding-places of the plankton-organisms, and as pointed out by Kofoid, their contributory function to the plankton of the river is at its maximum during *the decline of the floods.* It is during such times that vast accumulations of plankton-units are carried into the main stream.

Melosira varians, Fragilaria capucina, and *Cyclotella Kützingiana* are perennial plankton-organisms in the rivers we have examined.

X. General Comparison of British Lake-areas.

In summarising our present knowledge of the phytoplankton of the British lakes, it has been one of our first duties to obtain a record of the species which occur in the different areas. As these records have never previously been correlated, we have drawn up the following tabulated list of all the species observed in the phytoplankton of the British Islands.

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* Fritsch, *loc. cit.*, 1903, p. 637.

In this list those Algæ, which, so far as is known, are *exclusively confined to the plankton* are marked "P"; those which are *exclusively plankton-varieties* of species which frequently occur in other situations are marked "Pv"; and those species which are *more abundant in the plankton than elsewhere* are marked "p."

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
CHLOROPHYCEÆ.						
Œdogonium spp. (sterile)	×	×	×	×	×	×
,, *punctato-striatum*, De Bary	×					
Ulothrix zonata (Web. and Mohr), Kütz.	×	—	—	—	×	
,, *subtilis*, Kütz.	—	—	—	—	—	×
,, ,, var. *variabilis* (Kütz.), Kirchn.	×	×	×	×	×	
,, *moniliformis*, Kütz.	×	×				
Geminella interrupta, Turp.	×	—	×			
Myxonema subsecundum (Kütz.), Hazen.	—	—	×			
,, *tenue* (Ag.), Rabenh.	—	—	—	—	—	×
Microspora amœna (Kütz.), Lagerh.	×	—	×	×	×	
,, ,, var. *irregularis*, W. and G. S. West	—	—	×			
,, *abbreviata*, Rabenh.	—	—	—	×	×	
Mougeotia spp. (sterile)	×	×	×	×	×	×
,, *elegantula*, Wittr.	×	—	—	—	×	
Zygnema spp. (sterile)	×	—	×	×	×	
,, *ericetorum* (Kütz.), Hansg.	×					
Spirogyra spp. (sterile)	×	—	×	×	×	
Debarya glyptosperma (De Bary), Wittr.	—	×				
Gonatozygon monotænium, De Bary	×	×	×	×	×	
,, ,, var. *pilosellum*, Nordst.	×	—	×	×	×	
,, *Brébissonii*, De Bary	—	—	—	×		
,, *Kinahani* (Arch.), Rabenh.	×	×	×	×		
,, *aculeatum*, Hastings	×					
Genicularia elegans, W. and G. S. West (P)	×					
Spirotænia condensata, Bréb.	—	—	—	×		
Mesotænium macrococcum (Kütz.), Roy and Biss.	×	—	×			
Cylindrocystis diplospora, Lund.	×					
,, ,, var. *major*, West	—	—	—	—	×	
Netrium Digitus (Ehrenb.), Itzigs. and Rothe	×	×	×	×	×	
Penium Libellula (Focke), Nordst.	×	—	×			
,, ,, var. *interruptum*, W. and G. S. West	—	—	—	×		
,, *minutum* (Ralfs), Cleve.	×	×	×	—	×	
,, *margaritaceum* (Ehrenb.), Bréb., var. *irregularius*, W. and G. S. West	—	×				
,, *truncatum*, Bréb.	—	—	—	—	×	
Closterium abruptum, West	—	×				
,, *acutum*, Bréb.	×	—	—	—	×	×
,, *aciculare*, T. West, var. *subpronum*, W. and G. S. West (p)	×	—	×	—	—	×
,, *acerosum* (Schrank), Ehrenb.	—	×				
,, ,, var. *minus*, Hantzsch	×	—	×			

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Closterium Ceratium, Perty	—	—	—	—	—	×
,, *Cornu*, Ehrenb.	—	×				
,, *Cynthia*, De Not	—	×				
,, ,, var. *curvatissimum*, W. and G. S. West	×					
,, *decorum*, Bréb.	—	—	×			
,, *Dianæ*, Ehrenb.	×	—	—	—	×	
,, *Ehrenbergii*, Menegh.	×					
,, *incurvum*, Bréb.	—	×				
,, *Jenneri*, Ralfs	×					
,, *juncidum*, Ralfs	—	—	—	×		
,, *Kützingii*, Bréb.	×	—	×	—	×	
,, ,, var. *onychosporum*, W. and G. S. West (Pv)	×	—	×			
,, *Leibleinii*, Kütz	—	×	×			
,, *lineatum*, Ehrenb.	×					
,, *Lunula* (Müll.), Nitzsch.	—	×				
,, *macilentum*, Bréb.	×	×	—	×		
,, *moniliferum* (Bory), Ehrenb.	×					
,, *parvulum*, Näg.	×	—	×	—	×	
,, *pronum*, Bréb.	×	—	×			
,, *Pseudodianæ*, Roy	×	—	×			
,, *rostratum*, Ehrenb.	×					
,, *setaceum*, Ehrenb.	×	—	—	×	×	
,, ,, var. *elongatum*, W. and G. S. West (Pv)	×	—	—	—	—	×
,, *striolatum*, Ehrenb.	×					
,, *toxon*, West	×					
,, *tumidum*, Johnson	—	—	—	—	×	
,, *turgidum*, Ehrenb., forma *glabra*, Gutw.	×					
,, *Ulna*, Focke	×					
,, *Venus*, Kütz.	×					
Docidium Baculum, Bréb.	—	—	—	×		
Pleurotænium coronatum, Bréb.	×	—	—	×		
,, ,, var. *fluctuatum*, West	×					
,, *Ehrenbergii* (Ralfs), De Bary	×	—	×	×	×	
,, *nodosum* (Bail.), Lund.	—	—	—	×		
Tetmemorus Brébissonii (Menegh.), Ralfs	×					
,, *granulatus* (Bréb.), Ralfs	×	×	×	×	×	
,, *lævis* (Kütz.), Ralfs	—	×				
Euastrum affine, Ralfs	—	—	—	×		
,, *ampullaceum*, Ralfs	×					
,, *ansatum*, Raifs	×	×	×	×	×	
,, *bidentatum*, Näg.	×	×	×	×	×	
,, *binale* (Turp.), Ehrenb.	×					
,, *crassum* (Bréb.), Kütz.	×	—	—	×		
,, *denticulatum* (Kirchn.), Gay	×	×	×			
,, *Didelta* (Turp.), Ralfs	—	—	—	×		
,, *elegans* (Bréb.), Kütz.	×	×	×	×	×	
,, *gemmatum*, Ralfs	×	×				
,, *montanum*, W. and G. S. West	—	—	—	—	×	
,, *oblongum* (Grev.), Ralfs	×	×				
,, *pectinatum*, Bréb.	—	—	—	—	×	
,, ,, var. *inevolutum*, W. and G. S. West	×	×				
,, *pinnatum*, Ralfs	×					
,, *sinuosum*, Lenorm.	—	×				
,, *verrucosum*, Ehrenb.	×					

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Euastrum verrucosum, var. *reductum*, Nordst. (p)	×	×	×	×	×	
,, ,, var. *planctonicum*, W. and G. S. West (Pv)	×					
Micrasterias Americana, Ehrenb.	×					
,, *apiculata*, Menegh., var. *fimbriata*, Ralfs	×					
,, ,, var. *brachyptera* (Lund.), Nordst.	×					
,, *conferta*, Lund.	×					
,, *denticulata*, Bréb.	×	×	×	×	×	
,, *Jenneri*, Ralfs	×					
,, *Mahabuleshwarensis*, Hobson, var. *Wallichii* (Grun.), W. and G. S. West	×	×	—	—	×	
,, *Murrayi*, W. and G. S. West (P)	×					
,, ,, var. *triquetra*, W. and G. S. West (P)	×					
,, *papillifera*, Bréb.	×	×	×	×	×	
,, ,, var. *glabra*, Nordst.	—	—	—	×		
,, *pinnatifida* (Kütz.), Ralfs	×	—	—	×	×	
,, *radiata*, Hass. [= *M. furcata*, Ralfs] (p)	×	—	×	×	×	
,, *rotata* (Grev.), Ralfs	×	—	—	×		
,, *Sol* (Ehrenb.), Kütz. [= *M. radiosa*, Ralfs] (p)	×	×	×	×	×	
,, *truncata* (Corda), Bréb.	×	—	×	×	×	
Cosmarium abbreviatum, Racib.	×	×				
,, ,, var. *planctonicum*, W. and G. S. West (Pv)	×	—	×	×	×	
,, *angulosum*, Bréb., var. *concinnum* (Rabenh.), W. and G. S. West	—	×				
,, *bioculatum*, Bréb.	×	×	×	×	×	
,, *Blyttii*, Wille	×	—	—	—	×	
,, *Boeckii*, Wille	—	×				
,, *Botrytis*, Menegh.	×	×	×	×	×	×
,, ,, var. *tumidum*, Wolle	×					
,, ,, var. *depressum*, W. and G. S. West (Pv)	×	—	×			
,, *Brébissonii*, Menegh.	×	—	×			
,, *capitulum*, Roy and Biss., var. *grœnlandicum*, Börges. (p)	×	—	×	—	×	
,, *cælatum*, Ralfs	×					
,, *connatum*, Bréb.	×	—	×	—	×	
,, *contractum*, Kirchn.	×	—	×	×		
,, ,, var. *ellipsoideum* (Elfv.), W. and G. S. West	×	×	×	×	×	
,, *controversum*, West	×	—	—	—	×	
,, *Corribense*, W. and G. S. West (P)	—	—	×	×		
,, *costatum*, Nordst.	—	—	—	×	×	
,, *depressum* (Näg.), Lund. [= *C. Scenedesmus*, Delp.] (p)	×	×	×	×	×	
,, *depressum*, var. *achondrum* (Boldt), W. and G. S. West (p)	×	—	—	—	×	
,, *difficile*, Lütkem	×					
,, ,, var. *sublæve*, Lütkem	×	×	×	—	×	
,, *exiguum*, Arch.	—	—	—	×		
,, *formosulum*, Hoff.	×	×	—	—	×	
,, *globosum*, Buln.	—	—	—	×		
,, *granatum*, Bréb.	×	—	×			
,, ,, var. *subgranatum*, Nordst.	—	×	—	—	—	×
,, *humile*, Gay	×	×	×	×	—	×
,, *impressulum*, Elfv.	×					

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Cosmarium Kjellmanni, Wille, var. *grande*, Wille	×					
,, *Lundellii*, Delp., var. *æthiopicum*, W. and G. S. West	×					
,, *læve*, Rabenh., var. *septentrionale*, Wille	×					
,, ,, forma *octangularis* (Wille) nob.	×	×	×			
,, *Logiense*, Bissett	—	—	—	—	×	
,, *margaritatum*, Roy and Bissett	×	—	—	×		
,, *margaritiferum* (Turp.), Menegh.	×	×	×	×	×	
,, *Meneghinii*, Bréb.	—	×				
,, *moniliforme* (Turp.), Ralfs	×	—	×	×		
,, *ornatum*, Ralfs	×	—	×	×	×	
,, *orthostichum*, Lund.	—	—	—	×		
,, *ovale*, Ralfs	×	—	×			
,, *Phaseolus*, Bréb.	—	×				
,, *pseudopyramidatum*, Lund.	—	—	—	×		
,, *punctulatum*, Bréb.	×	×	×	—	×	
,, ,, var. *subpunctulatum* (Nordst.), Börges.	—	×	—	×		
,, *pyramidatum*, Bréb.	—	×	—	×		
,, *quadratum*, Ralfs, forma *Willei*, W. and G. S. West	—	—	—	—	×	
,, *Ralfsii*, Bréb.	×	—	—	×		
,, *reniforme* (Ralfs), Arch.	×	×	×	×	×	
,, *speciosum*, Lund.	—	×				
,, *subaversum*, Borge	×					
,, *subarctoum* (Lagerh.), Racib.	×	—	×	—	×	
,, ,, forma *punctata*, W. and G. S. West	—	×				
,, *subcostatum*, Nordst.	×	×				
,, *subcrenatum*, Hantzsch.	×	×	—	—	×	
,, *subcontractum*, W. and G. S. West (P)	×					
,, *subprotumidum*, Nordst.	—	×				
,, *subtumidum*, Nordst., var. *Klebsii* (Gutw.) W. and G. S. West (p)	×	×	×	×	×	×
,, *subundulatum*, Wille	—	—	—	×		
,, *subspeciosum*, Nordst.	—	×				
,, *Turpinii*, Bréb. (p)	—	×	×	—	×	×
,, *tetraophthalmum* (Kütz.), Menegh.	×	×	×			
Cosmocladium saxonicum, De Bary	—	—	×	×		
Xanthidium antilopæum (Bréb.), Kütz.	×	—	×	×	×	×
,, ,, var. *depauperatum*, W. and G. S. West (Pv)	×	×	×	×	×	
,, ,, var. *Hebridarum*, W. and G. S. West (Pv)	×	—	×			
,, ,, var. *læve*, Schmidle	×					
,, ,, var. *polymazum*, Nordst.	×					
,, ,, var. *triquetrum*, Lund. (p)	—	—	—	—	×	
,, *armatum* (Bréb.), Rabenh.	×	—	×	×	×	
,, ,, var. *cervicorne*, W. and G. S. West	×	—	—	×		
,, *cristatum*, Bréb.	×	—	×			
,, ,, var. *uncinatum*, Bréb.	—	—	—	—	×	
,, *fasciculatum*, Ehrenb.	×					
,, *subhastiferum*, West (p)	×	—	×			
,, ,, var. *Murrayi*, W. and G. S. West (Pv)	×	—	—	—	×	
,, *controversum*, W. and G. S. West, var. *planctonicum*, W. and G. S. West (Pv)	×					

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Xanthidium tetracentrotum, Wolle, forma, W. and G. S. West	×					
Arthrodesmus convergens, Ehrenb.	×	—	—	×	×	
„ *crassus*, W. and G. S. West (P)	×	—	×	—	×	
„ *Incus* (Bréb.), Hass.	×	—	×	×	×	
„ „ var. *longispinum*, W. and G. S. West (Pv)	×					
„ „ var. *Ralfsii*, W. and G. S. West, forma	×	—	—	×	×	
„ *octocornis*, Ehrenb.	×	—	—	×		
„ *quiriferus*, W. and G. S. West (P)	×					
„ *subulatus*, Kütz.	×					
„ *triangularis*, Lagerh.	×	×	—	×	×	
„ „ var. *subtriangularis* (Borge), W. and G. S. West (Pv)	×	×	×	—	×	
Staurastrum aculeatum (Ehrenb.), Menegh.	—	—	—	×		
„ *affine*, W. and G. S. West (P)	—	×				
„ *alternans*, Bréb.	—	×				
„ *anatinum*, Cooke and Wills (p)	×	×	×	×	×	×
„ „ var. *grande*, W. and G. S. West (p)	×	—	—	×		
„ „ var. *Lagerheimii* (Schmidle), nob. (p)	—	—	—	—	×	
„ „ var. *longibrachiatum*, W. and G. S. West (Pv)	×					
„ „ var. *pelagicum*, W. and G. S. West (Pv)	×	—	—	—	—	×
„ „ var. *truncatum*, West (p)	×	—	×	—	—	×
„ *angulatum*, West, var. *planctonicum*, W. and G. S. West (Pv)	×					
„ *apiculatum*, Bréb.	—	—	×	—	—	×
„ *Arachne*, Ralfs	×	—	×	×		
„ „ var. *curvatum*, W. and G. S. West (Pv)	×					
„ *Arctiscon* (Ehrenb.), Lund. (p)	×	—	×	×	×	
„ *aristiferum*, Ralfs	—	—	—	×		
„ „ var. *protuberans*, W. and G. S. West (Pv)	×					
„ *asperum*, Bréb.	×					
„ *Avicula*, Bréb.	×					
„ „ var. *subarcuatum* (Wolle), West	×	—	×	×		
„ *aversum*, Lund. (p)	×	—	×	×		
„ *Bienianum*, Rabenh.	×	—	×			
„ *boreale*, W. and G. S. West (P)	—	×				
„ *brachiatum*, Ralfs	×	×	×	×	×	
„ *Brasiliense*, Nordst., var. *Lundellii*, W. and G. S. West (p)	×	—	×	×	×	
„ *brevispinum*, Bréb. (p)	×	×	×	×	×	×
„ „ var. *altum*, W. and G. S. West (Pv)	×	—	×			
„ „ var. *obversum*, W. and G. S. West (Pv)	×					
„ *Cerastes*, Lund.	—	—	—	×		
„ *Clevei* (Wittr.), Roy and Bissett	×					
„ *conspicuum*, W. and G. S. West (P)	×					
„ *curvatum*, West (p)	×	×	×	×	×	
„ *cuspidatum*, Bréb.	—	—	—	—	—	×
„ „ var. *maximum*, W. and G. S. West (p)	×	×	×	×	×	×

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Staurastrum cuspidatum, var. *divergens*, Nordst.	×					
,, *cyrtocerum*, Bréb.	×					
,, ,, var. *compactum*, W. and G. S. West (Pv)	—	×				
,, *dejectum*, Bréb. (p)	×	×	×	×	×	×
,, ,, var. *inflatum*, West (p)	×	×	×	×	—	×
,, *denticulatum* (Näg.), Arch. (p)	—	—	×	×	×	
,, *Dickiei*, Ralfs	×	—	×			
,, *dilatatum*, Ehrenb., var. *obtusilobum*, De Not.	×	×	×			
,, *dorsidentiferum*, W. and G. S. West (P)	—	—	×			
,, *erasum*, Bréb. (p)	×	×	—	×	×	
,, *forficulatum*, Lund.	×					
,, *furcatum* (Ehrenb.), Bréb.	×	—	—	×		
,, *furcigerum*, Bréb. (p)	×	—	×	×	×	×
,, ,, forma *eustephana* (Ehrenb.)	—	—	—	×	×	
,, ,, forma *armigera* (Bréb.)	×					
,, ,, var. *reductum*, W. and G. S. West (Pv)	—	—	×			
,, *gracile*, Ralfs	×	×	×	×	×	×
,, ,, var. *nanum*, Wille	×	—	—	—	×	
,, ,, var. *cyathiforme*, W. and G. S. West, forma	×	—	×			
,, *grande*, Bulnh.	×	—	×	—	×	
,, *granulosum*, Ralfs	—	—	×	—	—	×
,, ,, var. *acutum* (Bréb.), W. and G. S. West	×					
,, *hexacerum* (Ehrenb.), Wittr.	—	×	×	×	×	
,, *hirsutum*, Bréb.	×					
,, *inflexum*, Bréb.	—	—	—	—	×	
,, *inelegans*, W. and G. S. West (P)	×					
,, *irregulare*, W. and G. S. West	—	—	—	—	×	
,, *jaculiferum*, West (biradiate vertical view) (p)	×	—	—	×	×	
,, ,, (triradiate vertical view) (p)	×	×	×	—	×	
,, ,, var. *excavatum*, W. and G. S. West (Pv)	×	—	×			
,, ,, var. *subexcavatum*, W. and G. S. West (Pv)	×					
,, *lævispinum*, Biss.	×					
,, *longispinum* (Bail.), Arch. (p)	×	—	×	×	×	
,, ,, var. *bidentatum* (Wittr.), W. and G. S. West (p)	×	—	—	×	×	
,, *lunatum*, Ralfs, var. *planctonicum*, W. and G. S. West (Pv)	×	×	×	×	×	
,, *Maamense*, Arch.	—	—	×			
,, *Manfeldtii*, Delp. (p)	—	×	×	—	×	
,, *megacanthum*, Lund. (p)	×	—	×	×	×	
,, ,, var. *Scoticum*, W. and G. S. West (Pv)	×					
,, *monticulosum*, Bréb., var. *bifarium*, Nordst.	×					
,, *mucronatum*, Ralfs, var. *subtriangulare*, W. and G. S. West (Pv)	×	—	—	×	×	
,, *muticum*, Bréb.	—	—	—	×	—	×
,, *Ophiuru*, Lund. (p)	×	—	—	—	×	
,, ,, var. *cambricum*, W. and G. S. West	—	—	—	×		
,, *orbiculare*, Ralfs	×					
,, ,, var. *depressum*, Roy and Bissett	—	×				

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Staurastrum paradoxum, Meyen	×	×	×	×	×	×
,, ,, var. *cingulum*, W. and G. S. West (Pv)	×	×	×	×	×	
,, ,, var. *longipes*, Nordst. (p)	×	×	×	×	×	×
,, *pelagicum*, W. and G. S. West (P)	—	×	×	—	—	×
,, *pilosum*, Näg.	—	×				
,, *polymorphum*, Bréb.	×	—	—	—	×	
,, *polytrichum*, Perty	×					
,, *pseudopelagicum*, W. and G. S. West (P)	×	×	×	×	×	
,, *punctulatum*, Bréb.	×	×	—	—	×	
,, *Saxonicum*, Buln.	—	×				
,, *Sebaldi*, Reinsch., var. *productum*, W. and G. S. West (Pv)	×	—	×			
,, ,, var. *ornatum*, Nordst.	—	—	×	×	—	×
,, *sexangulare* (Buln.), Rabenh. (p)	×	—	×	×	×	
,, ,, var. *supernumerarium*, W. and G. S. West (Pv)	×					
,, *sibiricum*, Borge	×					
,, *sublævispinum*, W. and G. S. West	×					
,, *subnudibrachiatum*, W. and G. S. West (P)	×					
,, *subgracillimum*, W. and G. S. West	×					
,, *subpygmæum*, West	×	—	×			
,, *teliferum*, Ralfs	×	×	—	×	×	
,, *tetracerum*, Ralfs	×	×	—	×		
,, *Tohopekaligense*, Wolle, var. *trifurcatum*, W. and G. S. West	×	—	×			
,, *tumidum*, Bréb.	×	—	—	×		
,, *verticillatum*, Arch.	×					
,, *vestitum*, Ralfs	—	—	—	—	×	
Spondylosium pulchrum (Bail.), Arch., var. *planum*, Wolle (p)	×	—	×	—	×	
Sphærozosma granulatum, Roy and Bissett	×	×	—	—	×	
,, *Aubertianum*, West	—	—	×			
,, ,, var. *Archerii* (Gutw.), W. and G. S. West (p)	×	—	—	×	×	
,, *excavatum*, Ralfs	—	—	×			
,, *vertebratum*, Ralfs	×	—	×	×	×	
Desmidium aptogonum, Bréb.	×	—	—	×	×	
,, *coarctatum*, Nordst., var. *cambricum*, West (p)	×	—	—	×		
,, *graciliceps* (Nordst.), Lagerh.	×					
,, *occidentale*, W. and G. S. West (P)	×					
,, *Pseudostreptonema*, W. and G. S. West (p)	—	—	×			
,, *Swartzii*, Ag.	×	—	—	×	×	
Gymnozyga moniliformis, Ehrenb.	×	—	×	×	×	
,, ,, var. *gracilescens*, Nordst.	×					
Hyalotheca dissiliens (Sm.), Bréb.	×	—	×	×	×	
,, ,, forma *tridentula*, Nordst.	×					
,, *Indica*, Turn. (p)	×	—	×	—	×	
,, *mucosa*, Ehrenb.	×	×	×	×	×	
,, *neglecta*, Racib. (p)	×	—	×	×	×	
,, *undulata*, Nordst.	—	—	×	×		
Volvox aureus, Ehrenb.	—	—	—	×	—	×
Pleodorina californica, Shaw	×					
Eudorina elegans, Ehrenb.	×	×	×	×	×	×
Gonium pectorale, Müll.	×	—	—	×		
Pandorina Morum (Müll.), Bory.	×	×				
Chlamydomonas pulvisculus, Ehrenb.	—	—	—	—	—	×

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Urococcus insignis, Kütz.	—	×				
Pediastrum Boryanum (Turp.), Menegh.	×	×	×	×	×	×
,, ,, var. *brevicorne*, A. Br.	—	—	—	—	—	×
,, ,, var. *granulatum*, Ralfs	—	×	×	—	—	×
,, ,, var. *longicorne*, Reinsch.	—	—	—	—	—	×
,, *duplex*, Meyen.	×	×	×	—	—	×
,, ,, var. *asperum*, A. Br.	—	—	×	—	—	×
,, ,, var. *clathratum*, A. Br. (p)	—	—	—	—	—	×
,, *glanduliferum*, Benn.	—	×	—	—	×	
,, *integrum*, Näg.	—	×				
,, *simplex*, Meyen.	×					
,, *Tetras* (Ehrenb.) Ralfs	×	—	—	—	—	×
Sorastrum Americanum (Bohlin), Schmidle	×					
,, *spinulosum*, Näg.	×					
Cœlastrum cambricum, Arch. (p)	×	—	×	—	—	×
,, *microporum*, Näg.	—	—	—	×	—	×
,, *Morus*, W. and G. S. West	×					
,, *reticulatum* (Dang.), Senn. (p)	—	—	×	—	—	×
,, *sphæricum*, Näg.	×	×	×			
Crucigenia quadrata, Morren	—	—	—	—	—	×
,, *rectangularis* (Näg.), Gay	×	—	×	—	×	
,, ,, forma *irregulare* (Wille)	×	×				
,, *Tetrapedia* (Kirchn.), W. and G. S. West (P)	—	—	—	—	—	×
Scenedesmus acutiformis, Schröder, var. *Brasiliensis* (Bohlin), W. and G. S. West	—	×				
,, *bijugatus* (Turp.), Kütz.	×	×	×	×		
,, ,, forma *arcuatus* (Lemm.), W. and G. S. West (p)	—	—	×			
,, *denticulatus*, Lagerh., var. *linearis*, Hansg.	×	×				
,, *obliquus*, Kütz.	×	—	—	—	—	×
,, *Hystrix*, Lagerh.	×					
,, *quadricauda* (Turp.), Bréb.	×	×	×	×	—	×
,, ,, var. *abundans*, Kirchn.	×	×	—	×	—	×
,, ,, var. *horridus*, Kirchn.	—	—	—	—	—	×
Dimorphococcus lunatus, A. Br.	×					
Dictyocystis Hitchcockii, Lagerh.	×					
Elakatothrix gelatinosa, Wille	—	—	—	—	×	
Ankistrodesmus biplex (Reinsch.), G. S. West	—	—	×			
,, *falcatus* (Corda), Ralls	×	×	×	×	×	×
,, ,, var. *acicularis* (A. Br.), G. S. West	×	×	×	—	×	×
,, ,, var. *mirabilis*, G. S. West...	—	×	—	—	—	×
,, ,, var. *spiralis* (Turn.), G. S. West (p)	—	×	—	—	—	×
,, ,, var. *spirilliformis*, G. S. West	×	—	—	×	×	
,, *Pfitzeri* (Schröder), G. S. West (P)	×	×	×	—	×	
Closteriopsis longissima, Lemm. (p)	—	—	×	—	—	×
,, ,, var. *tropicum*, W. and G. S. West	—	×				
Characium Debaryanum (Reinsch), De Toni	—	—	×			
Selenastrum gracile, Reinsch.	—	—	—	—	—	×
Kirchneriella lunaris (Kirchn.), Möb.	×					
,, *obesa*, W. and G. S. West (p)	×	×	×	—	—	×
Oocystis apiculata, West	—	×				
,, *crassa* (Wittr.)	×	×				
,, *lacustris*, Chodat (P)	—	—	×	—	×	
,, *Marssonii*, Lemm. (P)	—	—	×	—	—	×

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Oocystis parva, W. and G. S. West (p)	×	×	—	—	×	×
,, *solitaria*, Wittr.	×	—	×	—	—	×
Nephrocytium Agardhianum, Näg.	×	×	×	×	—	×
,, *lunatum*, West (p)	×	—	×	—	×	
Tetraëdron minimum, Hansg.	—	×	—	—	—	×
,, *cruciatum* (Wolle), W. and G. S. West	×					
,, *enorme* (Ralfs), Hansg.	—	—	×			
,, *limneticum*, Borge (P)	×					
,, *regulare*, Kütz.	×					
Dictyosphærium pulchellum, Wood (p)	×	—	×	×	×	×
,, *Ehrenbergianum*, Näg.	×	×				
Botryococcus Braunii, Kütz. [inclus. *Ineffigiata neglecta*] (p)	×	×	×	×	×	×
,, *protuberans*, W. and G. S. West (P)	×					
,, *sudeticus*, Lemm.	×					
,, ,, var. *planctonicus*, Lemm.	×					
Syhærocystis Schröteri, Chodat (P)	×	×	×	×	×	×
Tetraspora lacustris, Lemm. (P)	—	—	—	—	×	
Glœocystis gigas (Kütz.), Lagerh.	×	×	×	×	×	×
,, ,, var. *planctonicum*, W. and G. S. West (Pv)	—	—	×			
,, *infusionum* (Schrank), W. and G. S. West	×	—	×			
,, *vesiculosa*, Näg.	×	—	×			
Golenkinia paucispinosa, W. and G. S. West (P)	—	—	—	—	—	×
Richteriella botryoides (Schmidle), Lemm., forma *quadrata* (Lemm.), Chodat (P)	—	—	×	—	—	×
HETEROKONTÆ.						
Ophiocytium cochleare, A. Br.	×					
,, *bicuspidatum*, Lemm.	×					
,, *capitatum*, Wolle	×					
Tribonema affine (Kütz.), G. S. West	×					
Askenasyella conferta, W. and G. S. West (P)	×	—	×	—	—	×
Oodesmus Dœderleinii, Schmidle (P)	—	—	—	—	—	×
Chlorobotrys regularis (West), Bohlin	—	—	×	—	—	×
BACILLARIEÆ.						
Melosira crenulata, Kütz.	—	—	×	—	—	×
,, ,, var. *tenuis* (Kütz.), Grun.	—	—	—	—	—	×
,, *arenaria*, Moore	—	—	×	—	—	×
,, *granulata* (Ehrenb.), Ralfs (p)	×	×	×	×	×	×
,, *varians*, Ag.	—	—	×	—	—	×
Cyclotella compta, Kütz. (p)	×	×	×	—	×	×
,, ,, var. *affinis*, Grun.	×	×	×	×	×	×
,, *Kützingiana*, Chauvin	—	—	—	—	—	×
,, *Meneghiniana*, Kütz.	×	—	×			
,, *operculata*, Kütz.	×	—	×			
,, *Schröteri*, Lemm. (P)	—	—	×			
Coscinodiscus lacustris, Grun. (p)	—	—	—	—	—	×
Stephanodiscus Astræa (Ehrenb.), Grun. (P)	—	—	—	—	—	×
Rhizosolenia eriensis, H. L. Sm. (P)	×					
,, *longiseta*, Zach. (P)	×	—	×			
,, ,, var. *stagnalis*, Zach.	×					
,, *morsa*, W. and G. S. West (P)	×	—	×	—	×	
Tetracyclus lacustris, Ralfs	×	—	—	—	—	×

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Tabellaria fenestrata (Lyngb.), Kütz.	×	×	×	×	×	
,, ,, var. *asterionelloides*, Grun. (Pv)	×	×	×	×	×	×
,, *flocculosa* (Roth.), Kütz.	×	×	×	×	×	
Denticula tenuis, Kütz.	×	—	×	—	—	×
Meridion circulare, Ag.	—	—	—	—	—	×
Diatoma elongatum, Ag.	×	×	×	—	×	×
,, ,, var. *tenuis* (Ag.), V. H.	—	—	—	—	—	×
Fragilaria capucina, Desmaz.	—	—	—	—	×	×
,, *construens*, Grun.	×	—	—	—	—	×
,, *Crotonensis* (A. M. Edw.), Kitton (p)	×	×	×	—	×	×
,, ,, var. *contorta*, W. and G. West (Pv)	×					
,, *mutabilis* (W. Sm.), Grun.	×	×	×			
Amphipleura pellucida, Kütz.	×	×	×			
Synedra Acus (Kütz.), Grun.	×	×	—	×	×	×
,, ,, var. *angustissima*, Grun.	—	—	×			
,, ,, var. *delicatissima* (W. Sm.), V. H. (p)	—	—	—	—	—	×
,, *Lemmermanni*, W. and G. S. West (P)	—	—	×			
,, *pulchella*, Kütz.	×	×	×	—	×	
,, *radians* (Kütz.), Grun.	—	—	—	×	×	×
,, *Revaliensis*, Lemm.	—	—	×			
,, *Ulna* (Nitzsch), Ehrenb.	×	—	×	×	×	×
,, ,, var. *longissima* (W. Sm.), Grun.	—	—	—	—	—	×
,, ,, var. *splendens* (Kütz.), Grun.	—	—	—	—	—	×
Asterionella formosa, Hass. (p)	×	×	×	×	×	×
,, *gracillima*, Heib. (p)	×	—	×	—	×	×
Ceratoneis Arcus (Ehrenb.), Kütz., var. *Amphioxys* (Rabenh.), De Toni	×	—	—	—	×	
Eunotia biceps, (W. Sm.), G. S. West	×	—	—	—	×	
,, *Diadema*, Ehrenb.	×					
,, *gracilis* (Ehrenb.), Rabenh.	×	—	—	—	×	
,, *lunaris* (Ehrenb.), Grun.	×	—	×	×	×	
,, *major* (W. Sm.), Rabenh.	×	—	—	—	×	
,, *pectinalis* (Kütz.), Rabenh.	×	—	×	×	×	
,, ,, var. *bidens*, Grun.	×					
,, ,, var. *undulata*, Ralfs	×					
,, *tetraodon*, Ehrenb.	×					
Achnanthes coarctata, Bréb.	—	×				
,, *exilis*, Kütz.	×					
,, *flexella* (Kütz.), Bréb.	×	—	—	—	—	×
Stauroneis anceps, Ehrenb.	×					
,, *Phœnicenteron*, Ehrenb.	—	×	—	—	×	
Gomphonema acuminatum, Ehrenb.	×	—	—	×		
,, *constrictum*, Ehrenb.	×	—	—	—	×	
,, *geminatum*, Ag.	×	—	×	—	×	
,, *intricatum*, Kütz.	—	—	×	—	×	
,, ,, var. *Vibrio* (Ehrenb.), V. H.	—	×				
,, *olivaceum* (Lyngb.), Kütz.	—	×	—	—	×	
Cocconeis Pediculus, Ehrenb.	—	—	—	—	—	×
,, *Placentula*, Ehrenb.	×	—	—	—	—	×
Gyrosigma attenuatum (Kütz.), Rabenh.	—	×	×	—	—	×
,, *Spencerii* (Quek.), O. K.	—	—	—	—	—	×
Navicula alpina (W. Sm.), Ralfs	×	×				
,, *Brébissonii*, Kütz.	—	×				
,, *divergens*, Ralfs	×					
,, *elliptica*, Kütz.	×	×				
,, ,, var. *minima*, V. H.	×					

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Navicula gibba, Kütz.	×	—	—	—	×	
,, *Iridis*, Ehrenb., var. *affinis* (Ehrenb.), V. H.	—	×				
,, *major*, Kütz.	×	×	×	×	×	
,, *nobilis* (Ehrenb.), Kütz.	×					
,, ,, var. *Dactylus* (Ehrenb.), V. H.	×					
,, *pusilla*, W. Sm.	—	×				
,, *radiosa*, Kütz.	—	×	×	×	×	
,, *viridis*, Kütz.	—	×	—	—	×	×
Vanheurckia rhomboides, Bréb.	×					
,, ,, var. *Saxonica*, W. and G. S. West	×	×	×	×	×	
Cocconema cæspitosum (Kütz.), G. S. West	—	—	×			
,, *cuspidatum* (Kütz.), G. S. West	×					
,, *Cistula*, Ehrenb., var. *maculata*, Kütz.	×					
,, *cymbiforme*, Ehrenb.	×	×	×	—	×	
,, *Ehrenbergii* (Kütz.), G. S. West	×					
,, *gastroides* (Kütz.), nob.	×					
,, *gracile* (Raben.), nob.	—	—	—	×	×	
,, *lanceolatum*, Ehrenb.	×	×	×	—	×	
,, *ventricosum* (Ag.), nob.	×	—	—	—	×	
Amphora ovalis, Kütz.	×	×	×	—	×	×
Epithemia turgida (Ehrenb.), Kütz.	×	×	×	—	×	×
Rhopalodia gibba (Kütz.), O. Müll.	×	×	—	—	—	×
Nitzschia acicularis, W. Sm.	—	—	×	—	—	×
,, *dissipata* (Kütz.), Grun., var. *acuta* V. H.	—	×				
,, *linearis* (Ag.), W. Sm.	×	×	×			
,, *Palea* (Kütz.), W. Sm.	×	×	×	—	×	×
,, *Sigma* (Kütz.), W. Sm.	—	—	—	×	×	
,, *sigmoidea* (Ehrenb.), W. Sm.	×	—	×	—	—	×
Cymatopleura elliptica (Bréb.), W. Sm.	—	×	×	—	—	×
,, ,, var. *Hibernica* (W. Sm.) V. H.	—	—	—	—	—	×
,, ,, var. *rhomboides*, Grun.	—	—	×			
,, *Solea*, W. Sm.	×	—	×	—	—	×
Surirella biseriata, Bréb. (p)	×	—	×	×	×	×
,, *linearis*, W. Sm.	×	×	—	—	×	
,, *ovalis*, Bréb.	×	—	—	—	—	×
,, ,, var. *pinnata* (W. Sm.) V. H.	×					
,, ,, var. *angusta* (Kütz.) V. H.	×					
,, ,, var. *ovata* (Kütz.) V. H.	—	—	—	—	—	×
,, *robusta*, Ehrenb. (p)	×	×	×	×	×	
,, ,, var. *splendida* (Ehrenb.) V. H. (p)	×	×	×	×	×	×
,, *spiralis*, Kütz.	×	—	—		—	×
,, *turgida*, W. Sm.	—	—	×	—	—	×
Campylodiscus Hibernicus, Ehrenb.	×	×	×	—	—	×
MYXOPHYCEÆ.						
Hapalosiphon Hibernicus, W. and G. S. West	×					
Stigonema minutum, Hass.	×	—	×			
Nostoc microscopicum, Carm.	×					
Anabæna circinalis (Kütz.), Hansg. (p)	×	×				
,, *Hassallii* (Kütz.), Wittr.	—	—	×			
,, ,, var. *tenuis* (W. and G. S. West), Lemm.	—	—	×			
,, *Flos-aquæ* (Lyngb.), Bréb. (p)	×	—	×	×	×	×
,, *Lemmermanni*, P. Richter (P)	×	—	×	×	×	×
Lyngbya bipunctata, Lemm. (P)	—	—	—	—	×	

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
Lyngbya contorta, Lemm. (P)	×					
,, *Kützingii*, Schmidle, var. *distincta* (Nordst.), Lemm.	—	—	—	—	—	×
,, *limnetica*, Lemm. (P)	×	—	×	—	—	×
,, *Martensiana*, Menegh.	—	—	×			
,, *versicolor*, Gomont	×					
Oscillatoria tenuis, Ag.	×	×	×	×	×	×
,, *limosa*, Ag.	—	—	×	—	—	×
,, *Agardhii*, Gomont (p)	—	—	×	×	×	
,, *irrigua*, Kütz.	—	—	—	—	×	
,, *rubescens*, D. C. (P)	×	—	—	—	×	
Phormidium tenue (Menegh.), Gomont	—	—	×	—	—	×
Glœotrichia echinulata (Eng. Bot.), P. Richter (p)	×					
Glœothece linearis, Näg.	×	—	×			
Synechococcus major, Schroet.	×					
Merismopedia glauca (Ehrenb.), Näg.	×	×	×	×	×	
,, *æruginea*, Bréb. (p)	×	×	×	—	×	
,, *elegans*, A. Br.	—	×				
,, *punctata*, Meyen	×					
Merismopedia tenuissima, Lemm. (P)	—	—	×	×	—	×
Cœlosphærium Kützingianum, Näg. (p)	×	×	×	—	×	×
,, *minutissimum*, Lemm. (P)	×	—	×	×	—	×
,, *Nägelianum*, Unger.* (P)	×	×	×	×	—	×
,, *natans*, Lemm. (P.)	—	—	×			
Gomphosphæra aponina, Kütz.	—	—	×	—	—	×
,, *lacustris*, Chodat (P)	×	—	×	×	×	×
Aphanocapsa pulchra (Kütz.), Rabenh.	—	×				
Aphanothece saxicola, Näg.	—	—	×			
,, *clathrata*, W. and G. S. West (P)	—	—	×	—	—	×
Dactylococcopsis rhaphidioides, Hansg. (P)	—	—	×	—	—	×
Microcystis æruginosa, Kütz. (p)	—	—	×	—	×	×
,, *elabens* (Bréb.), Kütz.	×	×				
,, *icthyoblabe*, Kütz.	—	—	—	×		
,, *incerta*, Lemm. (p)	×	—	×	×	×	
,, *marginata*, Menegh.	×					
,, *Flos-aquæ* (Wittr.), Kirchn.	×	×				
,, *prasina* (Wittr.), Lemm. (p)	—	×	×	—	—	×
,, *pulverea* (Wood), Migula	—	—	—	—	×	
,, *roseo-persicina*, Kütz.	—	—	—	—	—	×
,, *stagnalis*, Lemm. (p)	×	×	×	—	×	×
Chroococcus cohærens, Näg.	×	×	×			
,, *helveticus*, Näg.	—	—	×			
,, *limneticus*, Lemm. (P)	×	×	×	×	—	×
,, ,, var. *subsalsus*, Lemm. (P)	—	—	×			
,, *minimus* (v. Keissler), Lemm.	×	×	—	—	×	×
,, *pallidus*, Näg.	×	×				
,, *turgidus* (Kütz.), Näg.	×	×	×	—	×	
PHÆOPHYCEÆ.						
Stichoglœa olivacea, Chodat (P)	×					
Phæococcus planctonicus, W. and G. S. West (P)	×	—	×			

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* It seems probable that this "species" is merely a form of *C. Kützingianum*, Näg.

Species.	Scottish lakes.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh lakes.	English lakes.	Lough Neagh (and L. Beg).
FLAGELLATA.						
Dinobryon bavaricum, Imhof. (p)	×	—	×			
„ *cylindricum*, Imhof. (p.)	—	—	×	×	×	
„ „ var. *pediforme*, Lemm.	×	—	—	×		
„ „ var. *palustre*, Lemm.	×	—	×			
„ „ var. *angulatum*, Lemm. (p)	×					
„ „ var. *divergens* (Imhof.), Lemm. (p)	×	×	×	×	×	×
„ „ var. *Schauinslandii*, Lemm. (p)	×	—	×			
„ *elongatum*, Imhof. (p)	×	—	×	×	×	
„ „ var. *undulatum*, Lemm. (p)	×	×	×	×	×	
„ *protuberans*, Lemm. (p)	×	—	—	×	—	×
„ *Sertularia*, Ehrenb.	×					
„ „ var. *thyrsoideum* (Chodat), Lemm. (p)	—	—	×	—	—	×
„ *sociale*, Ehrenb.	—	—	×			
„ *utriculus* (Ehrenb.), Klebs.	×					
Halobryon Lauterbornii, Lemm. (p)	×					
Synura uvella, Ehrenb.	—	—	—	—	×	
Mallomonas acaroides, Perty (p)	×	×	×			
„ *caudata*, Iwanoff	×	—	×			
„ *producta*, Iwanoff	—	—	×			
„ *longiseta*, Lemm. (P)	×	—	—	—	×	
Diplosigopsis frequentissima, Lemm. (p)	×	—	×	×		
Bicœca lacustris, J. Cl., var. *longipes*, Zach. (p)	—	—	×	×		
Cryptomonas erosa, Ehrenb.	—	—	×			
Lepocinclis ovum (Ehrenb.), Lemm., var. *punctato-striatum*, Lemm.	—	—	×			
Euglena viridis, Ehrenb.	×	×	×	×		
PERIDINIEÆ.						
Gymnodinium, sp.	—	—	×			
„ *paradoxum*, Schilling (p)	×	—	—	—	×	
„ „ var. *major*, Lemm. (Pv)	—	—	×			
Glenodinium pulvisculus (Ehrenb.), Stein (p)	×	—	×	—	—	×
Ceratium cornutum (Ehrenb.), Clap. and Lachm. (p)	×	×	×	×	×	
„ *curvirostre*, Huitf.-Kaas. (P)	×					
„ *hirundinella*, O. F. Müller (P)	×	×	×	×	×	×
Peridinium bipes, Stein	—	—	×			
„ *cinctum*, Ehrenb. (p)	—	—	×	—	—	×
„ *inconspicuum*, Lemm.	×	—	—	×	×	
„ *limbatum* (Stokes), Lemm. (p)	—	—	×			
„ *pusillum*, Schilling (p)	×					
„ *tabulatum* (Ehrenb.), Clap. and Lachm.	×	×	×	×	×	×
„ *umbonatum*, Stein	×					
„ *Westii*, Lemm. (P)	×					
„ *Willei*, Huitf.-Kaas. (P)	×	×	×	×	×	×

The above list includes the species of the lake-plankton only, and the Flagellata are very incompletely recorded.

Were the species of the helioplankton of large ponds, pools, and ditches also included, many of the Protococcaceæ (or Autosporaceæ) would have to be added, such as sundry species of *Chodatella, Lagerheimia, Golenkinia,* and other genera.

The following summary is instructive:—

	Species only.						Totals. Species and varieties of each group.	
	Scottish.	Orkneys and Shetlands.	W. and S.W. Ireland.	Welsh.	English lakes.	Lough Neagh (and L. Beg).	Species.	Varieties.
Chlorophyceæ (except Desmidiaceæ)	54	31	37	20	24	33	81	18
Desmidiaceæ	176	85	103	101	96	22	236	68
Heterokontæ	5	—	2	—	—	3	7	0
Bacillarieæ	63	37	47	18	41	41	94	22
Myxophyceæ	31	17	33	12	17	21	53	2
Phæophyceæ	2	—	1	—	—	—	2	0
Flagellata (except Peridinieæ)	12	4	13	6	4	3	18	7
Peridinieæ	11	4	10	5	6	5	15	1
Totals of species for each area, and grand total of both species and varieties for entire British phytoplankton	354	178	246	162	188	128	506	118
							Grand total.	

The grand total of 506 species and 118 varieties is sufficient evidence in itself that the British phytoplankton is exceedingly rich, and of this total 46 per cent. are species of the Desmidiaceæ.

Many of these constituents are, of course, adventitious or casual, consisting of littoral or bog species which are carried into the plankton by the rains and exist there only a short time before perishing. There are, however, many constituents which are exclusively limnetic in habit, and also others which are much more abundant in the plankton than in other situations. These have been discriminated in the foregoing list.

XI. GENERAL SUMMARY AND DISCUSSION ON THE DOMINANCE OF DESMIDS.

The tabulated list gives a very adequate idea of the Algal constituents of the British freshwater plankton, and also shows that many of these constituents are common to the four British lake-areas.

The British lakes combine to some extent the characteristic features of the Central European and Northern European lakes, but are on the whole more nearly akin to the latter. In addition they have peculiarities which tend to mark them off from either of those groups; for instance, the relatively high winter temperatures. Very many of these lakes never freeze, and most of the others only rarely become covered with ice, and then for comparatively brief periods. The summer temperatures are also comparatively low. The highest temperature we have recorded in Windermere in the course of twelve months' observations (including one of our warmest summers) was 14°·4 C. (58° F.), in Wastwater 17°·2 C. (63° F.), and in Ennerdale Water 15°·5 C. (60° F.). The highest temperatures we have obtained in the Welsh lakes were 17°·5 C. (63°·5 F.) in Llyn Ogwen and 18° C. (64°·5 F.) in Llyn Llydaw. The small sheet of water known as Llyn Elsie attained a summer (August) temperature of 19°·7 C. (67°·5 F.). The summer temperature of the Irish lakes rarely exceeds 18°·3 C. (65° F.). The average range of temperature in the Scottish lochs is from about 5° C. to 13° C., and the highest we have ourselves measured was 16°·6 C. (62° F.) in Loch Earn.

In all the four British lake-areas the water is soft, with only small quantities of dissolved lime, a peculiarity which accounts for the rarity of snails at the lake margins.

The phytoplankton is never of very great bulk, and it is quite exceptional for it to colour the water to any appreciable extent.

The periodicity of the phytoplankton is very variable in the different lakes. In some it is conspicuous, but in others it is not very well marked. Investigations at present in progress indicate that it is most conspicuous in the shallower lakes, and particularly in those at considerable altitudes, but at present our data are not nearly sufficient for generalisations to be made.

The MYXOPHYCEÆ play quite a secondary part in the plankton of the British lakes as compared with the Central European lakes. They are more abundant in some of the Irish lakes than in any of the others, and sometimes the phenomenon of "water-bloom" makes its appearance. This phenomenon, which is due to sudden and simultaneous maxima of a few of the limnetic species of Blue-green Algæ, is of very irregular occurrence in the British lakes, and is practically confined to sporadic appearances in some of the shallower-lakes.*

Oscillatoria tenuis is general but never abundant, and *Anabæna Lemmermanni* should be specially mentioned, as its spores form deep blue-green floating clusters, which sometimes give a decided colour to the surface water.

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* The phenomenon of "water-bloom" is better seen in some of the large pools and meres of the lowland parts of Britain than in any of the lake-areas. A good description of the "breaking of the meres" has been given by Phillips in 'Trans. Shropshire Archæol. and Nat. Hist. Soc.,' 1883, and earlier records were by Greville, Dickie, and Drummond.

Of the colonial unicells, *Cœlosphærium Kützingianum* (and the form of it known as *C. Nägelianum*), *Gomphosphæria lacustris*, and *Chroococcus limneticus* are the most important. *Microcystis* (*Clathrocystis*) *æruginosa* occurs more particularly in pools and small, shallow lakes, where the temperature of the surface water becomes relatively high in the summer.

In the Scottish and the Welsh lakes the Blue-green Algæ are decidedly scarce. This scarcity is to be attributed to the Alpine character of so many of these lakes, in which the maximum temperature of the surface water is relatively low.

The FLAGELLATA are well represented by various Peridinieæ, by *Mallomonas*, and by several species of *Dinobryon*. In many of the larger British lakes *Dinobryon* completely dominates the spring plankton, and a few colonies generally persist through the summer and the early part of the autumn. The most abundant species is *D. cylindricum*, and its var. *divergens* is equally common. In the smaller lakes *Dinobryon* is fairly general, but does not attain such great maxima as in the larger lakes. *Mallomonas acaroides* sometimes occurs in prodigious abundance, but lasts only a few weeks.

Of the Peridinieæ, *Ceratium hirundinella* is general, but it only occurs in large maxima in the smaller lakes. The variations in this organism have been well described and figured,* but there is one form in the lakes of the Outer Hebrides and the west of Ireland† which is apparently unknown in the plankton of the rest of Europe. In this curious form the first antapical horn is very much deflected to one side (*vide* fig. 3). Species of *Peridinium* often occur in very large quantity in the smaller lakes, and in the shallower of the large lakes. *P. tabulatum* is frequent, but *P. Willei* is general throughout the Scottish, Irish, and English lakes.‡ It occurs most abundantly in the small upland lakes (up to 1800 feet) with an Alpine character. *P. Westii* is exclusively confined to the Scottish lakes, where it appears to be frequent.

The BACILLARIEÆ are abundant in the British phytoplankton, but they rarely occur in such great quantities as in the Central European lakes, and scarcely ever form the vast maxima which periodically appear in so many of those lakes. Diatoms are fewest in the plankton of the Welsh lakes, forming only an average of 11 per cent. of the entire phytoplankton. This low percentage is probably due to the small numbers of adventitious species washed into the lakes from the mountain sides, and is possibly accentuated by the stony character of the lake margins and lake bottoms. They occur in the greatest variety in the Scottish and Irish lakes, probably owing to the large number of adventitious species washed into the lakes by the rains. The Pennate Diatoms are much the most numerous and conspicuous.

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* Lemmermann, in 'Archiv för Bot. utgifv. af K. Sv. Vet.-Akad.,' Bd. 2, 1904, No. 2, t. 2, f. 1—49; W. and G. S. West, in 'Roy. Soc. Edin. Trans.,' vol. 41, 1905, p. 494 (*c.* fig.); 'Roy. Irish Acad. Trans.,' vol. 33, sect. B, part 2, 1906, pp. 93, 94 (*c.* figs. 1—9); Bachmann in 'Archiv für Hydrobiologie und Planktonkunde,' Bd. 3, 1907, pp. 55—58, figs. I, I*a*, II.

† W. and G. S. West, in 'Roy. Irish Acad. Trans.,' *loc. cit.*, 1906, p. 94, f. 9.

‡ The distribution of *Peridinium Willei*, Huitf.-Kaas, extends from N. Italy, Ireland, England, and Scotland to Norway, Finland, the Faeroes, and Iceland.

Among the commonest forms are *Asterionella* (with a range of form and size which embraces both *A. formosa* and *A. gracillima*) and the two species of *Tabellaria*. *T. fenestrata* is much more abundant than *T. flocculosa,* except in the English lakes, where the reverse obtains. The chain-forms of *T. fenestrata* are the most frequently observed, but the star-dispositions (var. *asterionelloides*) are common except in the Welsh lakes. *T. fenestrata,* var. *asterionelloides,* is one of the dominating features of the late spring, the summer, and the early autumn plankton of many British lakes, and it exhibits great variability in the relative strength and breadth of the girdle view of the cells.* That these differences are of no varietal importance is proved by the occurrence of all intermediate stages.

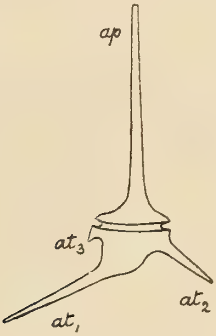

Fig. 3.—Peculiar Form of *Ceratium hirundinella*, O. F. M., in which the first antapical horn (at_1) is greatly deflected to one side. × 200.

We have not observed any star-dispositions of *T. flocculosa* in any of the British lakes, although such have been observed by Holmboe in Norway and by Wesenberg-Lund in Denmark (*T. flocculosa,* var. *pelagica,* Holmboe).

The numerous plankton-forms of *Asterionella* almost convince one that *A. formosa* and *A. gracillima* are merely states of the same species. No chains of *Asterionella* were observed in any of the lakes.

The genus *Fragilaria* is somewhat rare, and of the species which occur *F. capucina* is the commonest. *F. crotonensis* is very general in Scotland

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* *Cf.* W. and G. S. West, in 'Roy. Soc. Edin. Trans.,' vol. 41, 1905, Plate 2, figs. 1—3.

and Ireland, but is always scarce. We have not observed it in the English* or Welsh lakes. A variety of it—var. *contorta*—occurs in Loch Ruar, Sutherland, which is unique in the curious twisting of its exceedingly short filaments. This variety is not known from elsewhere.

Throughout all the British lake-areas, but more especially in the west of Scotland and the west of Ireland, species of the genus *Surirella* form a considerable and conspicuous part of the phytoplankton. The most frequent is *Surirella robusta*, var. *splendida*, which sometimes occurs in great abundance,† but *S. biseriata* and *S. linearis* are both general. In this respect the British lakes compare with the lakes of Central Africa, in which several plankton-species of *Surirella* are abundant.‡ In the Yan Yean Reservoir, Victoria, *S. robusta*, var. *splendida*, is also a constituent of the plankton.§ Wesenberg-Lund‖ states that various species of the genera *Surirella* and *Cymatopleura* occur in the plankton of principally alpine or shallow lakes in level country, and that they have been carried out by rivers and waves into the pelagic region, where they vegetate but for a short period and then perish. We find that in the British lakes, and also in those of Central Africa, the genus *Surirella* is frequently a true plankton-genus, and the various species which have been recorded both vegetate and multiply in the plankton to an extent we have rarely noticed in other situations.

Several of the Naviculaceæ occur with considerable regularity.

Centric Diatoms are relatively few and insignificant in the British lakes. *Melosira* is represented chiefly by *M. granulata* and *M. varians*. The latter is perennial in the plankton of British rivers. Species of *Cyclotella* are not abundant, and only in Lough Corrib, Galway, have we observed the curious gelatinous colonies which occur so frequently in some of the Central European lakes.

The genus *Rhizosolenia* is represented by two (and if the record of *R. eriensis* be correct, by three) species. *R. longiseta* is very rare, but *R. morsa* occurs in some of the lakes of all the British lake-areas. We have observed the resting-spores of this species in the June plankton of Thirlmere in the English Lake District.

No species of *Attheya* has yet been observed in any of the British lakes.

The CHLOROPHYCEÆ are well represented in the British lakes, more especially by the Desmidiaceæ. Apart from the latter, *Botryococcus Braunii* and *Sphærocystis Schroeteri* are the most general and abundant. Species of *Oocystis* are frequent, but never occur in quantity. *Dictyosphærium pulchellum* often occurs abundantly, but it also at times occurs in equal abundance in bogs.

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* This species occurs both in the plankton (helioplankton) and the benthos of the large pools in the Midlands of England.

† Consult W. and G. S. West, *loc. cit.*, Plate 1, figs. 1—4, and Plate 2, fig. 6 (photos).

‡ G. S. West, in 'Linn. Soc. Bot. Journ.,' vol. 38, 1907, p. 85.

§ G. S. West, in 'Linn. Soc. Bot. Journ.,' vol. 39, 1909, p. 17.

‖ Wesenberg-Lund, 'Plankton Investigations of the Danish Lakes,' Copenhagen, 1908, p. 42.

Eudorina elegans is fairly general, even in large lakes such as Lough Corrib, although it reaches its maximum abundance in small lakes.

Pediastrum Boryanum and *P. duplex* are frequent in the plankton of the shallower lakes, but *P. simplex* is very rare. Several species of *Cœlastrum*, *Scenedesmus*, and *Crucigenia* occur in many of the lakes, but never in quantity.

Species of *Zygnema*, *Spirogyra*, and *Mougeotia* occur in the plankton of most of the lakes, principally in the late spring and summer. They are usually the slender species of these genera, and are almost invariably sterile. In the smaller alpine lakes *Mougeotia* is often abundant, and forms no small part of the phytoplankton.* The curious coiled *Mougeotia*-filaments of some of the Scottish lochs have already been referred to. It would appear that the coiling is a limnetic character,† developed to augment the floating-capacity of the filament, and the fact of its presence is direct evidence that some of these solitary filaments of *Mougeotia* are adapting themselves to a life in the plankton.

THE MOST INTERESTING FEATURE OF THE BRITISH FRESHWATER PHYTOPLANKTON IS THE DOMINANCE OF DESMIDS. In 1903, and again in 1905, we showed that in contrast to any previously known plankton that of the Scottish lakes was unique in the abundance of its Desmids. Since then we have found that this dominance of Desmids is not confined to the lochs of the Scottish Highlands, but is a feature of the plankton of the four lake-areas of the British Islands, and that *the plankton of the western British lake-areas differs markedly from all other European plankton in the abundance of its Desmids.*‡

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* In the alpine lakes of the Pike's Peak Region, Colorado, Shantz states that species of *Spirogyra* and *Œdogonium* form a large part of the summer plankton (*vide* 'Amer. Microscop. Soc. Trans.,' March, 1907). Fragmentary filaments of various species of *Œdogonium* are also very frequent in the summer plankton of the British lakes.

† Consult G. S. West in 'Linn. Soc. Bot. Journ.,' vol. 38, 1907, pp. 85 and 86.

‡ Among European lakes, only those of Norway and certain parts of Sweden approach the British lakes in the possession of a conspicuous Desmid-flora in the plankton (consult Huitfeldt-Kaas, *loc. cit.*, 1906; and Lemmermann, in 'Archiv för Bot. utgiv. af K. Sv. Vet.-Akad.,' Bd. 2, 1904), and it should be mentioned that many of these plankton Desmids are identical with the British ones. Tanner-Fullemann has recorded the occurrence of a number of Desmids in the plankton of the Schoenenbodensee (*vide* 'Bull. de l'Herb. Boissier, 2me sér., t. 7, 1907), but the species which he records, when it is

In discussing this phenomenon of the rich Desmid-flora of the British freshwater plankton, it is necessary, in the first place, to briefly outline the general distribution of the Desmidiaceæ in the British Islands, *quite irrespective of the freshwater plankton.* We have studied the distribution of British Desmids in detail during the past 16 years, and the obvious fact, patent to anyone who chooses to collect these plants over extensive areas, is the *much greater richness of the Desmid-flora in the western areas of the country.* The eastern districts of England are exceedingly poor, but on passing from the newer Tertiary formations to the Older Palæozoic and Precambrian formations the Desmid-flora gradually increases in richness, attaining its maximum diversity in certain of the Precambrian areas.

The richest areas of all are the little boggy pools and smaller lakes of the Lewisian Gneiss of North-west Scotland and the Outer Hebrides, and similar areas on the Precambrian formations of Donegal, Mayo, and Galway. There are also several very rich localities in the English Lake District and North Wales, all on the Silurian and Ordovician with sundry Igneous intrusions. There are, in addition, two rich localities in the south of England, one on the Lower Greensand of Surrey (Thursley Common), and the other on the Middle Eocene of Hampshire (the New Forest). In both these localities there are deep, spongy bogs, with a fairly rich Desmid-flora, but at the same time it is a flora which falls far short of the much richer Desmid-floras of the Precambrian areas. We may add that these are not statements based upon a few casual observations, but upon a detailed examination of many thousands of collections made in all parts of the country, from the Shetland to the Scilly Islands, and from the east of England to the west of Ireland. It is also necessary to give some explanation of what is meant by a "rich" area. We do not apply the term "rich" to a mere abundance of Desmids, or even to the occurrence of a great quantity of 30 or 40 species, but only to those areas in which 150 to 200 (or even 300) species can be found in more or less abundance, including many of the rare species with a restricted distribution.

We have, therefore, as a foundation on which to base this discussion of

possible to be certain of his identifications, are those of shallow alpine and subalpine lakes, and not in any way comparable to the characteristic plankton species of the western British areas. Neither do we regard his records as constituting a "rich" Desmid-flora.

Among extra-European lakes, Victoria Nyanza has a conspicuous Desmid-flora in the plankton, and the Yan Yean Reservoir, Victoria, possesses a very rich Desmid-plankton, quite equal to the best of the British lakes, although with an entirely different association of species. In the latter case the drainage water is mostly from Silurian and Granitic outcrops, but it is not yet possible to make a definite statement concerning the drainage into Victoria Nyanza.

the dominance of Desmids in the plankton of the British lakes, a fairly complete and necessary knowledge of the general distribution of Desmids in the bogs, pools, etc., throughout the whole of the British Islands. The first point of importance is that the great majority of the British lakes (those constituting the western and north-western lake-areas) are all situated in the richest Desmid-areas in these Islands, or for that matter in Europe. It is, therefore, not in the least surprising that the plankton of these lakes should on the whole contain an abundance of Desmids. That the Desmids of the plankton should differ considerably from those of the bogs of the drainage areas—a matter discussed in a later part of this paper—does not affect the main question, *viz.*, *that the phytoplankton of these lakes possesses in many instances such an abundance of Desmids that it can be correctly described as a Desmid-plankton.*

Those facts which explain the abundance of Desmids in the bogs and bog-pools, among the mosses of the dripping rocks, and among the leaves of the submerged plants of the lake-margins, will likewise furnish the explanation of the abundance of Desmids in the plankton, as the plankton-Desmids have certainly originated from bog and swamp species, and others are being constantly recruited from the same sources. In endeavouring to discover the relationship between the conditions of environment and the richness of the Desmid-flora, two facts stand out very clearly :—

1. The rich Desmid-areas correspond very accurately with the areas of the old geological formations. They are mostly mountainous districts, with considerable outcrops of Igneous rocks.

2. These areas also correspond, but with less accuracy, to the areas of greatest rainfall.*

It is now necessary to enquire more closely into the relationships between the geological nature of the drainage-area, the rainfall, and the richness of the Desmid-flora.

We will first consider the *rainfall* of the areas in question. This is relatively heavy, varying from about 45 to upwards of 100 inches, and is due to two causes: first, to the fact that these areas are almost all near the west coast, being districts in which large mountains are situated in close proximity to the sea; and secondly, to the prevailing westerly and south-westerly winds. Such conditions naturally result in wet, mossy hill-sides, with numerous bogs. There is consequently much peaty water, rich in humic and other organic acids, in which submerged plants, such as *Utricularia minor*, *Sphagnum cuspidatum*, *S. subsecundum*, and other

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* Mr. James Murray (in 'Roy. Phys. Soc. Edin. Proc.,' vol. 16, 1905, p. 58) also points out that Sir John Murray had indicated this fact to him.

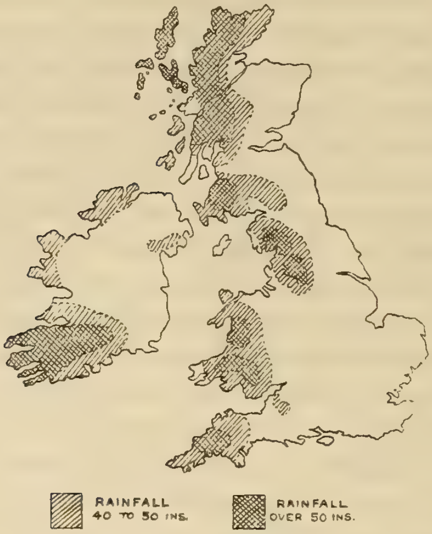
RAINFALL 40 TO 50 INS.
RAINFALL OVER 50 INS.

Fig. 4.

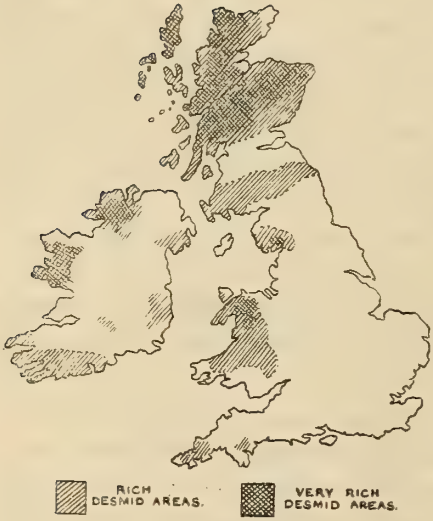
RICH DESMID AREAS.
VERY RICH DESMID AREAS.

Fig. 5.

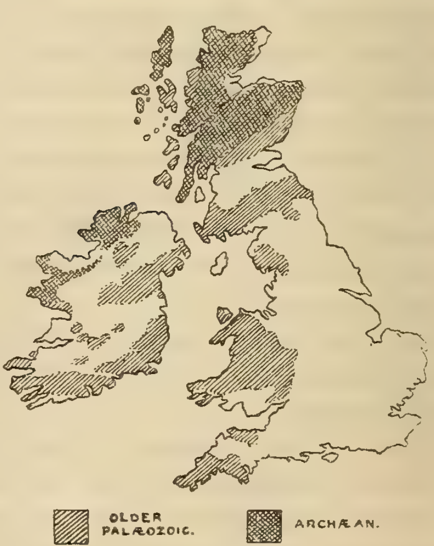
OLDER PALÆOZOIC.
ARCHÆAN.

Fig. 6.

Maps of British Islands, to show (fig. 4) areas with Rainfall over 40 inches; fig. 5, distribution of rich and very rich Desmid-areas, characterised by the western types mentioned (pp. 201 and 202); fig. 6, distribution of Older Palæozoic and Archæan rocks.

Note.—The Desmid-flora of the central and south-eastern counties of Ireland is very imperfectly known. Notice the falling off in the Desmid-flora of Skye in fig. 5, corresponding with the absence of Archæan rocks indicated in fig. 6.

aquatics thrive, and furnish all the requirements for the prolific growth of Desmids.

This seems a natural explanation of the occurrence of a rich Desmid-flora, and one which is accepted by Wesenberg-Lund* as the main cause of the phenomenon.

A detailed study of the distribution of Desmids has shown us, however, that the mere presence of suitable habitats is insufficient to account for the great richness of the Desmidiaceæ in certain areas of these Islands. Among the mountains of the Pennine Chain are some of the finest peat-bogs in the British Islands—in all outward appearances ideal spots for the occurrence of a rich Desmid-flora. Such are Cocket Moss, Austwick Moss, and others less well known. Again, on Thursley Common in Surrey, and in the New Forest, are bogs which are unsurpassed in Britain as habitats for the Desmidiaceæ. In all these localities the bogs are deep and dangerous, fed mostly by bottom springs, and furnish an ideal home for quantities of submerged *Sphagnum* and *Utricularia minor*. Desmids occur in countless millions among the larger aquatics ; in fact, collections made in these localities would be regarded as " rich " or " very rich " by those who had no experience of the western areas. *Utricularia minor*, which harbours some of the best of British Desmids among its leaves, flowers profusely in the localities mentioned, and no finer specimens can be obtained even in Mayo and Galway. Yet the Desmid-flora which occurs among the *Utricularia* in the above-mentioned localities is not to be compared with that which occurs in precisely the same environment in the western British areas, and, moreover, it contains none of the real British rarities.

How is it that an ideal locality such as Cocket Moss, which would be described as " rich in Desmids," contains practically none of those species which are both dominant and characteristic of the western areas ? The conditions are almost identical with those obtaining in the western bogs, and the rainfall is from 50 to 60 inches.

From a consideration of the above remarks it is obvious that some factor, other than mere abundance of rainfall and presence of ideal habitats, has a profound influence on the distribution of Desmids. This at once causes us to enquire into the statement that we have previously made,† viz., that *the rich Desmid-areas correspond geographically with the Precambrian and Older Palæozoic outcrops* (*together with the intrusive Igneous material*). (Consult figs. 5 and 6.)

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* Wesenberg-Lund, ' Plankton Investigations of the Danish Lakes,' Copenhagen, 1908 p. 281.

† *Vide* p. 197 of the present paper.

In the first place, it has been recently pointed out* that the association of the rich Desmid-areas with the older strata is (in the British Islands) most probably due in part to the antiquity and consequent hardness of the rocks. The mountainous regions which have resulted from those changes in the earth's surface which have produced folding and contortion, and from the resistance of these old, compressed rocks to subaerial denudation, are not only directly responsible for the rainfall owing to their geographical position, but are themselves most suitable for the formation of peat-bogs. In these areas Desmids flourish, and therefore, so far as the British Islands are concerned, the richness of the Desmid-flora bears a distinct relationship to the antiquity of the geological formations of any area under consideration. It seems probable that the determining factor is a chemical one. It is certainly something more than mere suitability of habitat, otherwise how is the relative poorness of the extensive bogs of the more recent formations to be explained ?†

It is very probable that the chemical composition of the water plays an important part in determining this distribution. That the chemical factors are quite apart from the occurrence of brown, peaty water, is evident from the poorness of the Desmid-flora of so many peat-bogs, and also from the fact that the best and richest Desmid-floras only occur in clear water with no obvious peaty characters. Wesenberg-Lund is incorrect when he states that Desmids *chiefly* thrive in "brown water rich in humic acids."‡ Some of them certainly do, and often profusely, but these are generally the common, ubiquitous species which have almost a world-wide distribution. The great majority, including most of the western British types, prefer clear water with little peat. An excess of the brown peaty material is distinctly unfavourable. The plankton-Desmids also occur much more abundantly in the clear lakes than in the brown peaty ones.

Although it appears so probable that chemical factors determine the distribution of many Desmids, no definite information on this point has yet been obtained. The drainage water which has percolated through the old formations (rocks and soil) may possibly contain minute quantities of something in solution which greatly favours the development of certain types of Desmids, and may be directly responsible for their restricted distribution.§

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* G. S. West, in 'Linn. Soc. Bot. Journ.,' 1909, vol. 39, p. 10.

† How is it, also, that the peaty bogs and ditches of the fens of the east of England, such as are left of them, are poorer in the Desmidiaceæ than any other part of the British Islands ?

‡ Wesenberg-Lund, *loc. cit.*, 1908, pp. 280 and 281.

§ There would be nothing remarkable in this, as diatoms thrive and build up their siliceous cell-walls in water containing silica in such minute quantities that ordinary chemical analysis reveals no trace of it.

In reference to the above remarks, James Murray* has stated that "another theory is that the lochs which are richest in Desmids are only found in the older geological formations, but this does not accord with facts, as I find that such lochs occur in all the formations from the Lewisian to the Tertiary, at least; and it will, I think, be found that some of these lochs lie entirely in glacial deposits." It should be pointed out that if glacial drift is excluded none of the lake-areas are even near the British Tertiary formations. Many of the lakes have doubtless been formed in Tertiary times, and if they are spoken of as "Tertiary lakes" it must be distinctly understood that *they are situated in drainage-basins on the old formations.* Those lakes which Murray has termed "Tertiary lakes" are certain of the Scottish lochs which are surrounded by and rest upon more or less extensive sheets of glacial drift. It must be distinctly borne in mind that most of this glacial drift has been derived from the old rocks, and what is very much more important, that the drainage into such lakes consists mostly of water which has partly traversed the exposed outcrops of the old rocks of the surrounding hills and mountains, and partly percolated through them. Therefore, any peculiarities which may be characteristic of the drainage water from the old formations, are also equally characteristic of the water of such lakes.

The point of primary importance is that the greater part of the drainage water of such a lake has traversed the older rocks, and possesses those peculiarities which so far as we can see account for the Desmid-flora not only of its plankton, but of its littoral region and also of the surrounding bogs. It is immaterial *when* the lake was formed, or whether its bed be one of glacial drift or of old rocks.

IN THE BRITISH ISLANDS THE REALLY RICH DESMID-FLORAS, CONTAINING MANY OF THE WESTERN BRITISH TYPES, ARE ONLY FOUND IN THOSE AREAS WHICH COMBINE THE MOST SUITABLE HABITATS (*such as are found on boggy hill-sides with an abundant rainfall*) WITH A DRAINAGE-WATER DERIVED FROM GEOLOGICAL FORMATIONS OLDER THAN THE CARBONIFEROUS.

We would suggest a very exact chemical investigation of the water of bogs and lakes in different areas as a possible means of throwing further light upon this question. It has been suggested that the absence of lime is the determining factor in the abundance of Desmids, and it may possibly have much to do with the restricted distribution of the western British types.

There are a large number of western types of British Desmids, of which the most important are included in the following list:—

Gonatozygon aculeatum, Hastings; *Spirotænia trabeculata*, A. Br.; *Penium*

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* James Murray, *loc. cit.*, 1905, p. 58.

adelochondrum, Elfv.; *P. Clevei*, Lund.; *Tetmemorus Brébissonii* (Menegh.), Ralfs, var. *minor* De Bary.

Micrasterias radiata, Hass.; *M. conferta*, Lund.; *M. pinnatifida* (Kütz.), Ralfs; *M. apiculata* (Ehrenb.), Menegh., and var. *brachyptera* (Lund.), W. and G. S. West.

Euastrum pictum, Börges. forma; *E. Turneri*, West; *E. aboense*, Elfv.; *E. intermedium*, Cleve; *E. pingue*, Elfv.; *E. validum*, W. and G. S. West.

Docidium undulatum, Bail.; *Pleurotænium eugeneum* (Turn.), W. and G. S. West.

Cosmarium bipunctatum, Börges.; *C. capitulum*, Roy and Biss., var. *grœnlandicum*, Börges.; *C. Corribense*, W. and G. S. West; *C. commissurale*, Bréb., var. *crassum*, Nordst., *C. distichum*, Nordst.; *C. didymoprotupsum*, W. and G. S. West; *C. entochondrum*, W. and G. S. West; *C. monomazum*, Lund., var. *polymazum*, Nordst.; *C. obsoletum* (Hantzsch), Reinsch; *C. perforatum*, Lund.; *C. pseudexiguum*, Racib.; *C. pseudopyramidatum*, Lund., var. *stenonotum*, Nordst.; *C. quadridentatum*, W. and G. S. West; *C. quadrifarium*, Lund.; *C. retusum* (Perty), Rabenh.; *C. retusiforme* (Wille), Gutw.; *C. Smolandicum*, Lund., var. *angustatum*, West; *C. sexnotatum*, Gutw., var. *tristriatum* (Lütkem.), Schmidle; *C. subquadrans*, W. and G. S. West; *C. subretusiforme*, W. and G. S. West; *C. synthlibomenum*, West; *C. taxichondriforme*, Eichl. and Gutw.; *C. tenue*, Arch.; *C. tumidum*, Lund.; *C. venustum*, Bréb., var. *hypohexagonum*, West; *C. viride* (Corda), Josh.; *C. zonatum*, Lund.

Staurastrum Arctiscon (Ehrenb.), Lund.; *St. aversum*, Lund.; *St. bacillare*, Bréb.; *St. Brébissonii*, Arch.; *St. Brasiliense*, Nordst., var. *Lundellii*, W. and G. S. West; *St. Cerastes*, Lund.; *St. Clevei* (Wittr.), Roy and Biss.; *St. conspicuum*, W. and G. S. West; *St. corniculatum*, Lund.; *St. curvatum*, West; *St. dorsidentiferum*, W. and G. S. West; *St. elongatum*, Barker; *St. erasum* Bréb.; *St. forficulatum*, Lund.; *St. grande*, Bulnh.; *St. lævispinum*, Biss.; *St. longispinum* (Bail.), Arch.; *St. maamense*, Arch.; *St. megalonotum*, Nordst.; *St. natator*, West; *St. Ophiura*, Lund.; *St. Picum*, W. and G. S. West; *St. quadrangulare*, Bréb.; *St. setigerum*, Cleve; *St. sexangulare* (Bulnh.), Rabenh.; *St. spiniferum*, West; *St. subgracillimum*, W. and G. S. West; *St. cosmospinosum* (Börges.), W. and G. S. West; *St. Duacense*, W. and G. S. West; *St. Hibernicum*, West; *St. jaculiferum*, West; *St. cornutum*, Arch.

In addition to the above, there are a number of species which are extremely rare in a few of the richest localities outside the western areas, whereas in the latter they are generally distributed and many of them prodigiously abundant. Such are:—

Spirotænia acuta, Hilse; *Netrium oblongum* (De Bary), Lütkem., var.

cylindricum, W. and G. S. West; *Penium exiguum,* West; *Closterium Ulna,* Focke; *Tetmemorus minutus,* De Bary; *Micrasterias Sol,* Ehrenb.

Euastrum crassum (Bréb.), Kütz., var. *scrobiculatum,* Lund.; *E. crispulum* (Nordst.), W. and G. S. West; *E. inerme,* Lund.; *E. pinnatum,* Ralfs; *E. pulchellum,* Bréb.; *E. sublobatum,* Bréb.; *E. ventricosum,* Lund.

Cosmarium connatum, Bréb.; *C. Debaryi,* Arch.; *C. decedens,* Reinsch; *C. elegantissimum,* Lund.; *C. annulatum* (Näg.), Arch., var. *elegans,* Nordst.; *C. Hammeri,* Reinsch; *C. isthmium,* West; *C. Nymannianum,* Grun.; *C. ovale,* Ralfs; *C. parvulum,* Bréb.; *C. pseudamœnum,* Wille; *C. pseudopyramidatum,* Lund.; *C. pseudoconnatum,* Nordst.; *C. sphœroideum,* West; *C. subundulatum,* Wille; *C. variolatum,* Lund.

Staurastrum aculeatum, Ehrenb.; *St. anatinum,* Cooke and Wills; *St. Arnellii,* Boldt; *St. furcatum,* Ehrenb.; *St. inconspicuum,* Nordst.; *St. arachne,* Ralfs; *St. lanceolatum,* Arch.; *St. oxyacanthum,* Arch.; *St. pungens,* Bréb.; *St. scabrum,* Bréb.; *St. aristiferum,* Ralfs; *St. pileolatum,* Bréb.; *St. pterosporum,* Lund.; *St. subscabrum,* Nordst.

Having discussed the most important facts concerning the general distribution of Desmids in the British Islands, we can now return to the abundance of the Desmids in the British freshwater phytoplankton.

We have shown which areas of these Islands possess the rich Desmid-floras, and when one considers that the British lakes are almost all situated in these western areas, it is not very surprising that they possess a plankton containing numerous Desmids. Neither is it surprising that many of these should be the western types, provided that these western types are capable of withstanding the conditions of a limnetic life.

THEREFORE WE CONSIDER THAT THE DESMIDS OF THE BRITISH FRESHWATER PHYTOPLANKTON ARE DUE LARGELY, AND THE WESTERN TYPES ENTIRELY, TO THE SITUATION OF THE LAKES IN THE RICH DESMID-AREAS OF THE OLD FORMATIONS.

The antiquity of the geological formations is not a special factor in the occurrence of the numerous *plankton Desmids,* but in the occurrence of *Desmids as a whole.* The presence of numerous Desmids in the plankton of the lakes follows as a matter of course.

One does not expect an abundance of Desmids in the plankton of the large Swiss lakes. They are situated in poor Desmid-areas, and in North Switzerland the geological formations are for the most part too recent.

Most of the Central European lakes are situated in areas relatively poor in Desmidiaceæ. In Denmark the formations are Cretaceous and Jurassic, largely overlain by drift, and similarly the lakes of Northern Germany are situated on immense areas of drift, overlying comparatively recent formations. Hence the dearth of Desmids in the lakes. On the other hand, the Scandinavian lakes are situated on the old formations, and contain an abundance of Desmids, many of which are identical with those of the British lake-areas.

The Desmids of the plankton have without doubt originated from the Desmid-community of the surrounding area, although in most cases there is little resemblance between the plankton-community and that which can be observed in the surrounding drainage-basin. There is an almost complete absence from the surrounding peat-bogs and dripping rocks of those species which are most conspicuous and abundant in the plankton. The common Desmids of the bogs are only found in the limnetic region of the lakes as casual or adventitious constituents, and therefore the great majority of the Desmids brought by the rains into this limnetic region, with its new conditions of life, find it impossible to maintain their further existence, and rapidly perish. The plankton-community as a whole, as shown by Wesenberg-Lund, is a very ancient one, and this is further confirmed in the case of the British lakes by the existence of this distinct community of plankton-Desmids. We have already stated* that many of the plankton-Desmids "have existed under these pelagic conditions for a long time, as there is every indication of this in the modifications some of them have undergone, and in the species and varieties which are at present only known to occur in the plankton."

During the vast period in which Desmids have been washed by the rains from their bog habitats into the lakes, a specific selection has taken place, certain species having adapted themselves, with or without slight morphological changes, to a limnetic existence. Only those have survived which were able to withstand the new conditions.

One of the principal conditions necessary for existence in the new life would be the ability to float in the surface waters. In some species, more especially in the discoid species of *Micrasterias*, this necessity has brought about no morphological alteration; and in others, amongst which are certain species of *Cosmarium* and *Staurastrum*, there is again no change of external form, but a copious development of surrounding mucilage. In many others morphological changes have occurred, mostly in the further development of those characters which have proved of most avail in the struggle against sinking. It is thus that spines and processes have been greatly increased in length, so that many of the plankton-forms are the longest-spined forms known.† Certain species of *Staurastrum* and *Arthrodesmus* best exhibit this great development of spines. The species of *Closterium* found in the British lake-plankton are mostly adventitious constituents, and are never abundant.

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* W. and G. S. West, in 'Roy. Soc. Edin. Trans.,' vol. 41, 1905, p. 512.

† In the ordinary habitats of Desmids, and in the former habitats of plankton-species spines are commonly found well developed. This armature has probably two functions,

Nearly all the plankton-Desmids are summer and autumn constituents, and the majority of them attain their maximum abundance in September and October, during the slight fall after the maximum summer temperature.

Neither plankton-Desmids nor those which occur in other situations undergo any seasonal form-variations. This we have conclusively proved by the examination of large numbers of periodic collections of these plants. This is merely what one would expect, as environmental form-changes in Desmids occupy long periods of time. As regards the plankton, the variations in the conditions of buoyancy during the year in the surface waters of a lake are not so great as the environmental differences between the habitats in which the same species of Desmid will thrive.

Large numbers of both the plankton and bog species survive the winter in the vegetative condition, and the formation of zygospores appears to be very rare.

In Diatoms it is known that the seasonal form-variation, when it occurs, is in the colony and not in the individual, but colonial Desmids are much fewer and much less abundant than colonial Diatoms.

Lastly, we would comment upon the *cosmopolitanism of the freshwater plankton-community.* This is generally true except for the Desmids. Wesenberg-Lund* states that the numerous plankton researches in the Central European lakes have been unable to demonstrate any special, geographically localised plankton-communities. He remarks† that the "freshwater plankton-communities, in contrast to all other communities on land or water, everywhere contain the same types, nearly everywhere the same species." As regards the Desmid-flora, however, these statements do not hold good. *Wherever there are lakes with a rich Desmid-flora in the plankton, there one also gets a more or less definitely localised plankton-community.* It has been stated‡ that the Desmidiaceæ show more decided geographical peculiarities than any other group of Freshwater Algæ, notwithstanding the fact that a large number of them are cosmopolitan and ubiquitous all the world over. These geographical peculiarities occur

one of anchoring the individual to its environment, and the other to serve as a protection against Desmid-eating animals. Whether the more elongated spines of the plankton-species likewise serve a similar function of protection against the depredations of the plankton Rotifers and Entomostraca is a point which requires further investigation.

* Wesenberg-Lund, *loc. cit.*, 1908, p. 293.

† *Loc. cit.*, p. 313.

‡ G. S. West, in 'Linn. Soc. Bot. Journ.,' vol. 38, 1907, p. 82; W. and G. S. West, in 'Ann. Roy. Bot. Gard., Calcutta,' vol. 6, part 2, 1908, p. 176.

in the Desmid-community of the plankton quite as much as in the general Desmid-community of the surrounding country. In fact, they appear to be well marked.*

Even with the meagreness of our present knowledge we can recognise three distinct plankton-communities of Desmids, which can at once be distinguished from each other, and which form a most interesting comparison. These are (1) the Desmids of the British (and to a certain extent of the Scandinavian) plankton, (2) the Desmids of the plankton of Victoria Nyanza, and (3) the Desmids of the Victorian plankton (as exemplified by the Yan Yean Reservoir). There are doubtless several other distinct plankton-communities of Desmids, notably in the South American and the Indo-Malayan regions. There are marked geographical peculiarities in the general Desmid-community of these regions, and should any of the lakes be found on investigation to possess a Desmid-plankton, it is highly probable that many of the species will possess their proper geographical character.

* In considering this question it should be borne in mind that Desmids in the vegetative condition cannot be blown about by the wind, as even partial desiccation is almost invariably fatal. Also, that in most species zygospores are very rarely formed, and that no zygospore has yet been observed of any of the typical plankton-species. The only Desmids which appear to survive a partial desiccation are certain species of *Cylindrocystis* and *Penium*, and possibly of *Mesotænium*.

On the Presence of Hæm-agglutinins, Hæm-opsonins, and Hæmolysins in the Blood obtained from Infectious and Non-Infectious Diseases in Man. (*Second Report.*)

By Leonard S. Dudgeon, F.R.C.P. Lond.

(Communicated by Dr. F. W. Mott, F.R.S. Received February 18,—Read March 4, 1909.)

(From the Pathological Laboratories, St. Thomas's Hospital.)

On July 31, 1908, my preliminary communication on this subject was received by the Royal Society and was read on November 12, 1908.

In this report attention was drawn to certain phenomena occurring when normal and immune human serum was allowed to act in the presence of normal and immune human blood cells. The whole of the investigations were carried out with human blood obtained from various infective and non-infective diseases in man. The technique adopted in all experiments was referred to in detail, and will not be described in the present communication. The most important results were obtained in the examination of the agglutinative properties of the blood when an interaction took place between serum and red cells. It was shown that auto-agglutination was a rare phenomenon, but iso-agglutination was common. In some instances hæm-agglutination occurred when the immune serum and normal red cells were mixed together; in other cases the effect was produced by the interaction of normal serum and immune red cells. In many examples of this reaction the agglutinated red cells were altered in shape and size, especially when the clumps were exceptionally large. Attention was drawn to the distinction between agglutination of red blood corpuscles and agglutination of rouleaux. Saturation experiments were performed, and the specificity of the various reactions was demonstrated. Immune serum from cases of infection with the bacillus typhosus was rendered specifically inactive by saturation with suitable red cells, although the bacterial agglutinins remained.

Attention must be drawn to the fact that it was stated in the preliminary report that agglutination was not observed when normal serum was added to normal red cells, either of the same individual or from another healthy person, with one exception. A considerable amount of further investigation along this line has shown that the second part of this statement requires alteration, as will be subsequently referred to.

Experiments on phagocytosis of red blood corpuscles were in the main negative, although numerous methods were adopted. It was in only one case out of the entire number investigated that the phagocytosis was well marked. In the majority of instances the experiments on hæmolysis led to negative results. In one case of acute poisoning, of unknown nature, physiological salt solution (0·85-per-cent. pure sodium chloride) was found to be able to hæmolyse the immune red cells. In two cases of pneumonia evidence of hæmolysis was tested for in the blood examined *in vitro*. In one instance well-marked auto-hæmolysis occurred, in the other iso-hæmolysis. Some degree of iso-hæmolysis was noted occasionally in the experiments with the blood obtained from other diseases, and in one further case auto-hæmolysis.

In the present communication many more interesting results have been obtained in various experiments conducted along the same lines and much more additional information acquired. It will be necessary at the commencement to give a list of the various diseases that have been investigated, and to point out that exactly the same care has been exercised in proving the accuracy of the diagnosis.

14 cases of typhoid fever; 9 cases of tuberculosis (mostly acute pulmonary); 3 cases of acute peritonitis due to appendicitis; 14 cases of anæmia (7 of pernicious anæmia), myelæmia, congenital cholæmia, and examples of anæmia secondary to various well recognised conditions; 7 cases of acute pneumonia; 6 cases of acute streptococcus infection; 2 cases of epilepsy; 4 cases of syphilis; 2 cases of diabetic coma; 3 cases of malignant disease; 5 cases of obscure toxæmia, including one case of paroxysmal hæmoglobinuria.

Hæmolysins.

The technique adopted was exactly that already referred to in the preliminary report, with the additional observations obtained by allowing the mixture of serum and red cells to be in contact in ice for 1 hour previous to incubation at 37° C. for a similar period, followed by exposure to ice for several hours. These final results in all cases were similar, so that this method was abandoned.

In the previous paper, the following conclusion was arrived at:—"In all these experiments on hæmolysis, it was only occasionally that the hæmolytic action was distinctly shown; in the majority of instances no hæmolysis occurred."

Typhoid Fever.—No hæmolysis was noted in any of the cases of typhoid fever which were examined. The results obtained in this disease, as in many others, were probably due to the fact that *normal serum* was only occasionally employed in these experiments. Out of the total number of cases investigated in the present series—14—hæmolysis was demonstrated in nine instances—a very high proportion.

It has been noted in these experiments that when the *immune serum* causes *hæmolysis* of *normal red cells* that the cases are of the *severe toxic type* and *terminate fatally*. It is true that the number of cases investigated is too small to lay too much emphasis on this observation, but it is unquestionably important.

In the first case, immune serum caused marked hæmolysis in the presence of normal red cells. A gradual diminution in the reaction occurred from the mixture which contained 75·0 per cent. of serum down to that which had a serum content of 25·0 per cent., and in one instance 12·5 per cent. In the second case, which terminated fatally, an exactly similar result was noted; in the third case, the blood examined a few days before death and that obtained at the *post-mortem* examination gave a similar result, with the exception that the serum obtained during life had a slightly greater potency.

In the remaining cases in which hæmolysis occurred, it was due to the action of normal serum on the immune red cells, and the reaction took place with about the same serum content as with the immune serum and normal red cells in the fatal cases. In all the experiments immune serum and immune red cells failed to react; in fact, no other type of hæmolysis occurred beyond that referred to.

The blood of a case of paroxysmal hæmoglobinuria has been examined during the acute attack and during the interval. The blood drawn from the finger in the usual way failed to show tinging of the serum with hæmoglobin when the usual precautions were adopted. It can be stated briefly that during the height of an acute attack, which is caused by severe cold, that the blood undergoes auto-hæmolysis, but when tested as the attack subsides, although the man is obviously ill, no auto-hæmolysis can be demonstrated. The immune serum also fails to hæmolyse normal red cells, although it agglutinates them strongly, and the immune urine fails to hæmolyse the immune red cells. When, however, normal serum is added in definite measured volumes to the immune red cells, hæmolysis occurs, although not to any very great extent, and hæmolysing agglutinins can be similarly demonstrated. It would seem that the red cells themselves are principally affected—a point still further illustrated by the phagocytic experiments to be subsequently referred to. The blood of two cases of diabetic coma obtained after death failed to hæmolyse the immune red cells, but, as in the fatal cases of typhoid fever, hæmolysed normal red cells and agglutinated them strongly; in each instance hæmolysis occurred down to a serum content in the mixture of 37·5 per cent.

Splenic Extract.—Owing to observations made on the splenic cells in certain diseases in which much phagocytosis of red cells occurs, it suggested itself that in the splenic juice might be found a substance or substances intimately concerned with phagocytosis, hæmolysis, and certain other phenomena, although absent or present in an infinitesimal degree in the blood serum. The spleen from one of the cases of diabetic coma was extracted in a suitable machine, the thick lumpy extract filtered at high speed, and the centrifugalisation repeated with the upper layer of extract which separated during the first stage. This final sticky, but *thin* extract, was used for testing its hæmolytic value on normal red cells and the auto-immune cells as in the case of serum investigation.

Four series of experiments were made by allowing the splenic extract to act on auto-immune cells and normal cells, incubating the mixtures at 37° C. for one hour, and then in the ice safe over night. A similar double series of tubes prepared in an identical manner were first placed in ice overnight, then at 37° C. for two hours, and finally in ice for several hours. All results were identical. It must be pointed out here that the hæmolytic mixture was proved to be free from bacteria. Now while the immune blood serum in this case failed to hæmolyse the immune red cells, and only reacted slightly in the presence of normal cells, the action being limited to a serum content of 37·5 per cent., yet the splenic extract was equally and strongly hæmolytic to normal and the auto-immune cells. The action was complete with a splenic content of 25 per cent., and limited to a mixture containing 0·25 per cent. of splenic extract. In a case of pernicious anæmia, on the other hand, neither the immune serum nor splenic extract hæmolysed the immune red cells. It is impossible, on the results of two experiments, to refer more fully to these investigations on the hæmolysing action of the splenic extracts on red blood cells, but from the work of Hédin and others we know that the spleen does contain proteid enzymes of considerable potency.

The blood was investigated very fully in seven cases of pernicious anæmia, and of all diseases it might well be imagined that auto-hæmolysis would be demonstrated in this one. In the preliminary communication it was pointed out that auto-hæmolysis could not be proved. In this paper a similar result must be recorded, neither could hæmolysis be induced by allowing the immune serum to act on normal red cells. It was found, however, that the immune serum had the same power to hæmolyse guinea-pigs' red cells as normal human serum possessed. In three instances normal serum was capable of hæmolysing the pernicious red cells to a marked degree: in each example hæmolysing agglutinins were present, while in two out of the three cases phagocytosis of the immune red cells occurred to a striking degree in the presence of normal serum and normal leucocytes.

In one instance, although normal serum hæmolysed the immune red cells during the patient's life, yet the action failed to take place with the immune cells after death. In every case the serum had the same striking greenish yellow coloration previously referred to.

In a case of acute primary syphilis, normal human serum was found to cause a high degree of hæmolysis in the presence of immune red cells, and the immune serum had a somewhat similar although less marked action on normal red cells. A similar result occurred in a case of acute pneumonia. The immune serum, when acting on normal red cells and the normal serum on immune cells, showed a high and equal degree of hæmolysis, while the immune serum and immune red cells failed to react.

These are the most important and striking results in the hæmolytic experiments, but it is necessary to emphasise the fact that many more positive results have been obtained than are quoted in the previous report, and those which are of special importance occurred in the blood reactions of the typhoid fever patients.

In many of these experiments on hæmolysis, and similarly in the agglutination and phagocytic investigations, the chief interest centres around the *immune red cells.* It is especially concerning these bodies that further research is being conducted.

Hæm-agglutinins.

Attention has already been drawn to the fact that the same technique has been adopted in all these experiments as previously given in detail in the preliminary communication.

I must again emphasise the rarity of auto-agglutination of red blood corpuscles. With one striking exception—that of a black man—no true example has been met with. This case will be subsequently referred to in detail.

In every instance, without exception, iso-agglutination occurred whenever iso-hæmolysis was demonstrated. While the agglutination experiments were being examined microscopically it was generally possible to detect the hæmolytic agglutinins from the pure agglutinins. In the former, many red cells at the margin of the clumps were smaller than those centrally placed and greatly resembled oil droplets. In the most striking instances, free hæmoglobin could be seen and numerous ghosts.

Iso-agglutination was of common occurrence in many diseases, as stated in the preliminary communication. This was especially so in typhoid fever and tuberculosis.

Hæm-agglutinins in Normal Blood.

Attention has already been drawn to a statement made in the preliminary communication on this subject. Since then a wide study of hæm-agglutinins in normal blood has been made. It has now been proved that although a certain sample of normal serum may fail to react with one or two specimens of washed normal red cells, yet, if sufficient examples of normal red blood cells are presented to that specimen of normal serum, agglutination will be found to occur in a certain proportion of instances, while some samples of normal serum agglutinate most specimens of normal red cells presented to them. With our wider knowledge we can state that auto-agglutination does not occur, and that the hæmolytic agglutinins have not met with, that type of agglutination which is of such interest in typhoid and certain other infections. These results are important because they compel one to realise that the demonstration of hæm-agglutination is not necessarily a reaction of pathological significance.

Auto-agglutination.

A negro from the West Indies, who had not been out of England for over ten years, showed a blood possessing remarkable properties. He was considered to be a case of tertiary hepatic syphilis. When blood escaped from his tissues from a single puncture, the red cells could be seen to be clumped in the plasma, and when the bleeding was continued into citrated saline the red cells fell to the bottom of the tube in enormous clumps. This is the only instance met with of a blood showing *spontaneous agglutination*, and auto-agglutination of such a high degree. In the preliminary communication, attention was called to the condition of the blood in a case of long standing epilepsy : here well-marked auto-agglutination was present, but not spontaneous agglutination, and the degree of agglutination was nothing like to the same extent as was obtained with the blood of the negro. On the addition of the immune serum to normal red cells a high degree of agglutination occurred, and it was the true hæmolytic type.

It had previously been shown that if an immune serum is diluted with normal saline, it rapidly loses its power of agglutinating red cells, quite out of proportion to what is observed in the case of the bacterial agglutinins. This sample of immune serum, however, clumped normal red cells when diluted to the extent of 1 in 10 almost as well as the undiluted serum, and some clumping occurred in a dilution of 1 in 500. This is the only instance out of the whole series of sera which have been examined in which such a phenomenon was noted. When the negro's blood was examined about a month later, neither spontaneous nor auto-agglutination was present, and normal serum failed to react with these cells, while when the immune serum and normal red cells were mixed hæmolytic agglutination occurred as before.

Typhoid Fever.—Auto-agglutination was never found to occur. The iso-agglutinins are of great interest. In every instance marked agglutination of red cells occurred when either the immune cells were added to normal serum or normal cells to immune serum. In a certain number of experiments both phenomena occurred. The fact that there was absence of agglutination when immune serum was added to normal red cells, although the serum clumped typhoid bacilli, can readily be explained by the absence of any relationship between bacterial and hæm-agglutinins. It was especially in these experiments that the hæmolytic agglutinins were noted after serum and red cells had been in contact for one hour at 37° C.

Attempts were made to extract "hypothetical agglutinins" from the red blood corpuscles in those diseases in which a marked reaction took place between the immune red cells and normal serum, but without success. The red cells were powdered in a mortar with finely broken glass, and the mixture centrifuged at high speed. The fluid resulting from this procedure was added to suitable red cells, but in no instance did agglutination occur. Other methods with a similar object in view were found to be equally futile.

Specific Agglutinins.

A considerable amount of further work has been completed to emphasise the specificity of the hæm-agglutinins. The same technique has been adopted, except that it has been shown that it is unnecessary to incubate the active mixtures of red cells and serum for several hours as on previous occasions. Two or three hours has since proved to be sufficient, while it has been shown that a considerable degree of this special reaction can be removed when the active red cells and serum are mixed well together for *two or three minutes,* not incubated, but immediately centrifuged, and the resulting fluid tested in the usual manner. It was found that although slight agglutination with the clear fluid and suitable red cells could be demonstrated, yet the greater proportion of it had been removed.

This experiment will serve to show the effect of a temperature of 37° C. for one and a quarter hours on the specific agglutinins:

Immune Serum (Typhoid) + Normal red cells.
Extreme degree of agglutination present.

Same serum saturated with same undiluted normal red cells for one and a quarter hours at 37° C.; mixture centrifuged.

Clear fluid + Normal red cells.
No agglutination.

When the immune typhoid serum was saturated with the typhoid red cells in a similar manner to the above, the clear fluid agglutinated normal red cells as before. It must be pointed out here that although saturation of a serum with suitable red cells will render the serum specifically inactive, and that saturation with inactive red cells will have no effect, yet, if the serum is capable of agglutinating the active red cells from two different diseases, saturation with one class of red cell may fail to render the serum inactive for the other red cells. Further work is being done on this important point at the present time.

The Effect of Saturating an Immune Serum with Melanin.

It has been shown conclusively in this paper that if you saturate typhoid serum with suitable red cells you remove the hæm-agglutinins, but leave the bacterial, and *vice versâ.*

Other experiments were undertaken for the purpose of ascertaining whether it would be possible to remove or greatly diminish the hæm- or bacterial-agglutinative action of the serum by means of melanin as Mr. Shattock and I have found to occur in experiments on phagocytosis.* Immune serum (typhoid) was saturated with sterile melanin for 12 hours at 37° C. The resulting fluid obtained after centrifugalisation was still capable of agglutinating typhoid bacilli and suitable red cells as the unsaturated serum, and also to the same degree.

The Effect of Saturating a Serum with Heated Red Cells.

In the experiments about to be referred to, it has been shown that it is possible to remove the agglutinative properties of a serum by saturating it with heated red cells. The red cells for this purpose are thoroughly washed in the usual manner, and definite proportions from the deposit of red cells obtained at the end of the final process of centrifugalisation are put in suitable tubes with a small quantity of physiological salt solution; the tubes are sealed, placed in a tube of water at a definite temperature, and then put into a water bath at the same temperature as the tube of water. By this means, and this

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* S. G. Shattock and L. S. Dudgeon, 'Roy. Soc. Proc.,' B, vol. 80, 1908, "Observations on Phagocytosis by means of Melanin and the Comparison of the Opsonic Index with the Hæmo-phagocytic Index."

Table to illustrate certain Specific Agglutinative Properties of Immune Serum from a Case of Typhoid Fever.

1.	2.	3.	4.	5.	6.
Simple bacterial agglutination. Results (typhoid serum and typhoid bacilli)—	Hæm-agglutination. Results (typhoid serum and normal red cells)—	Test 1 repeated subsequent to the saturation of the immune serum with normal red cells for 1¼ hours at 37° C.	Test 2 repeated subsequent to the saturation of the immune serum with typhoid bacilli for 1¼ hours at 37° C.	Test 2 repeated subsequent to the saturation of the immune serum with normal red cells.	Test 1 repeated subsequent to the saturation of the immune serum with typhoid bacilli.
Dil. 1/20, very marked reaction. Dil. 1/100, ditto. Dil. 1/500, slight reaction. Dil. 1/1000, very slight reaction.	One volume of serum and one volume of a 5-per-cent. suspension of washed red cells in normal saline. Red cells agglutinated in enormous clumps.	Dil. 1/20, very marked reaction. Dil. 1/100, ditto. Dil. 1/500, slight reaction. Dil. 1/1000, very slight reaction.	Red cells agglutinated in enormous clumps.	No agglutination occurred.	No agglutination occurred.
All tubes incubated for one hour at 37° C. in a water bath in the horizontal position, then all night in the perpendicular, at room temperature.	All tubes incubated for one hour at 37° C. and then examined.				

The typhoid bacilli employed in these tests were killed by exposure to 60° C. for 1 hour.

only, is it possible for the cells to reach the temperature indicated by the thermometer. We know that it is stated that if a serum is heated to 65° C., it loses certain properties which the unheated serum possesses; as a matter of fact, if human serum is kept at the temperature of 65° C. for 15 minutes in the manner indicated, it is rendered useless for serum investigation owing to the fact that it is coagulated.

In the experiment about to be referred to, certain immune red cells were heated at 60° C. in the manner indicated for 50 minutes, and at the end of that time formed a thick dark mass. Pneumonic serum and these red cells gave a high degree of agglutination before saturation, but after the serum had been saturated with the heated red cells for one and a quarter hours at 37° C., it was rendered inactive. This shows that, although the red cells were physically so altered, yet they still possessed a specific function. Similar results were obtained in another series of experiments in which the red cells were heated to 60° C. for one hour.

The Relation of the Bacterial and Hæm-agglutinins.

Several series of experiments have been completed, and the results clearly demonstrate that the bacterial and hæm-agglutinins are distinct. Typhoid serum, especially a serum possessing high agglutinative properties, is especially suitable for the purpose.

As will be seen from the accompanying table, the highest degree of specificity exists, and every experiment gave similar results. The saturation experiments were carried out in every detail in an identical manner.

Phagocytosis.

In summarising the technique and the results of the experiments on phagocytosis in my preliminary paper, it was stated that "It is unnecessary to refer at great length to the very large number of experiments made, as it was only in a few instances that phagocytosis was pronounced. In quite a number of instances, whether the serum was unheated, or diluted, or heated, or whether normal or immune leucocytes were employed, the degree of phagocytosis was infinitesimal." There was only one instance out of the total number investigated in which the phagocytosis of red blood corpuscles was a conspicuous feature. In the present communication striking instances of red cell phagocytosis have been recorded on several occasions, these different results mainly depending upon the nature of the diseases which are now being investigated. It is especially marked in such acute diseases as typhoid fever, while in pernicious anæmia, where it might be expected to occur, it is usually absent. Care must always be exercised in arriving at conclusions on this subject, because, unless the film preparations are made with special precautions, red cells which are apparently engulfed are really extra-cellular, and also, if the hæmolysins are active in the samples of serum employed, the red cells which are engulfed may be either pale or difficult to recognise, and, therefore, phagocytosis may be unknown. In a case of typhoid fever with typhoid pyuria a high degree of phagocytosis was noted. Normal leucocytes + immune red cells + normal serum showed no less than 37 red cells engulfed by 50 leucocytes, while when the normal serum was diluted with immune serum the phagocytosis was correspondingly reduced. In this instance normal serum caused marked agglutination of immune red cells and also hæmolysed them; in another case of typhoid the phagocytic test carried out in an identical manner showed 26 red cells engulfed by 50 leucocytes, the normal serum in this case also hæmolysed and agglutinated the immune red cells, while in a still further example the immune serum + normal red cells and immune leucocytes gave an active result—50 leucocytes contained 39 red cells; when normal serum was added to the immune red cells the phagocytosis was well marked, but very much less so; here also the immune serum strongly agglutinated normal red cells, but did not hæmolyse them. In certain other instances of typhoid infection some degree of phagocytosis was noted, but only of an indifferent type. In the case of paroxysmal hæmoglobinuria the immune red cells in the presence of normal serum and normal leucocytes showed a high degree of phagocytosis, 50 leucocytes contained 39 red cells. Experiments conducted with other variations in the cells and serum gave negative results.

The normal serum in these experiments, hæmolysed, agglutinated and incited phagocytosis of the immune red cells, but in different degrees, the most marked results occurring in the agglutination experiments, the least striking were in the hæmolytic. In a case of untreated secondary syphilis, experimental results showed absence of hæmolysis and agglutination, but complemented immune serum incited a high degree of phagocytosis of the immune red cells. Perhaps the most striking example of phagocytosis of the red blood corpuscles occurred when the serum from a case of epilepsy was added to normal red cells in the presence of normal leucocytes, 50 white cells contained 46 red blood corpuscles. The immune serum also strongly agglutinated these cells. Many more examples of this variety of phagocytosis have occurred in the numerous experiments which have been made, but the most interesting and striking results are those which I have recorded.

All instances in which hæmolysis occurs when the red cells and serum interact must tend to limit the accuracy of the phagocytic experiments, because so many red cells which have been engulfed by the leucocytes are ghosts and, no doubt, have been too severely hæmolysed to be recognisable.

In the concluding remarks in the preliminary paper on hæm-opsonins, it was stated that "The experiments referred to in this communication entirely agree with the observations of Barratt and Keith, conducted with the blood sera and cells from the lower animals. There was nothing to show that the agglutination, opsonic, or hæmolysing properties of normal or immune sera on red blood corpuscles have any direct relation to one another."

From the investigations which have been made for this report, some of which have been referred to, it has been noted that a serum may hæmolyse, agglutinate, and incite phagocytosis of certain red cells, but although it may be capable of agglutinating and hæmolysing certain red cells, yet the degree of phagocytosis which is present may be negligible, or phagocytosis may be present without the other phenomena; on the other hand, a serum which has a high agglutinative value is more likely to give a somewhat similar incitor reaction than otherwise, although not necessarily.

I have again to thank Mr. H. A. F. Wilson for his invaluable assistance, and also Dr. Athole Ross and Mr. Irvine, all co-workers in my laboratories.

The Theory of Ancestral Contributions in Heredity.

By Karl Pearson, F.R.S.

(Received March 19,—Read April 22, 1909.)

Under the above title a paper has recently appeared by Mr. A. D. Darbishire in the 'Roy. Soc. Proc.,' vol. 81, B, p. 61 *et seq.*, giving further experimental evidence with regard to the inheritance of certain characters in peas. The paper is an interesting one, but the method adopted is not, I venture to think, capable of answering the problem which the author set himself. It has been supposed by some Mendelians that the theory of inheritance summed up in the "law of ancestral heredity" was in some way invalidated by investigations such as Mr. Darbishire's, and that opinion consciously or unconsciously seems to be expressed in the paper just referred to. The law of ancestral heredity is embraced in the following statements:—

(i) In a population breeding without assortative mating the regression line for offspring on any ancestor is linear.

(ii) The correlations between offspring and the successive grades of ancestry form a progression diminishing geometrically as we ascend to distant grades; and

(iii) The general relation of an individual to his ancestry can be closely expressed by the multiple correlation formula.

In a memoir published in the 'Phil. Trans.,' vol. 203, pp. 53–86, I showed that these principles held for material obeying Mendel's laws—in particular (i) and (ii) hold for the simple case of alternative characters such as are said to occur in the case of peas.

The only instance that I am aware of in which ancestry does not matter is that in which the geometrical progression is of the form:

$$\rho, \rho^2, \rho^3, \ldots .$$

I treated this case at length in the 'Phil. Trans.,' vol. 187, A, pp. 304–6 (1896), remarking that the grandparents were quite indifferent, when the parents had been selected. Unfortunately, this is not true when the correlation coefficients are

$$\frac{1}{3}, \ \frac{1}{2}\times\frac{1}{3}, \ \frac{1}{2^2}\times\frac{1}{3}, \ \frac{1}{2^3}\times\frac{1}{3}, \text{ etc.,}$$

as is the case with the somatic correlations on the Mendelian theory. In other words, ancestry does matter in the latter theory. What is the explanation, therefore, of the apparent contradiction between such experiments as those of Mr. Darbishire and the theoretical development of the Mendelism which they profess to establish?

It does not seem hard to account for the divergence. Experiments such as those of Mr. Darbishire do not deal with a population as a whole, and consider the contributions to the next generation of all its components supposed to be mated at random. I feel quite certain that if Mr. Darbishire makes the requisite crosses in due proportions, and does not weight with differential fertility, he will find that ancestry does matter. That it does matter is just as good a proof of Mendelism as Mr. Darbishire's proof in the simpler case that it has not any effect. If he fails to find its influence, then he will have refuted Mendelian theory.

To illustrate my point, take a population distribution which would follow from crossing two pure races with respectively dominant and recessive characters represented by the letters D and R. Suppose the hybrids to cross at random, then the population will remain absolutely stable with the permanent formula

$$(\mathrm{DD})+2\,(\mathrm{DR})+(\mathrm{RR}).$$

Now suppose this to cross with itself or with

$$(\mathrm{DD})+2\,(\mathrm{DR})+(\mathrm{RR}).$$

Table I gives the scheme of offspring with their parents. This population of 16 individuals of 6 different types of parentage now crosses with itself. The result is a population of 256 individuals showing 15 types of grandparentage. This is exhibited in Table II. If Mr. Darbishire's principle that ancestry is of no importance were correct, then the differences in type of these grandparents would not be of any significance.

Table I.

Parents.	Offspring.		
	DD.	DR.	RR.
DD, DD	1	—	—
DD, DR	2	2	—
DD, RR	—	2	—
DR, DR	1	2	1
DR, RR	—	2	2
RR, RR	—	—	1

Table II.

Grandparents.					Offspring.		
DD.	DR.	RR.	+	−	DD.	DR.	RR.
4	—	—	4	—	1	—	—
3	1	—	4	—	6	2	—
3	—	1	3	1	2	2	—
2	2	—	4	—	13	10	1
2	1	1	3	1	8	14	2
2	—	2	2	2	1	4	1
1	3	—	4	—	12	16	4
1	2	1	3	1	10	28	10
1	1	2	2	2	2	14	8
1	—	3	1	3	—	2	2
—	4	—	4	—	4	8	4
—	3	1	3	1	4	16	12
—	2	2	2	2	1	10	13
—	1	3	1	3	—	2	6
—	—	4	—	4	—	—	1

Now Table II may be examined from several standpoints. We may first consider the gametic constitutions of the grandparents and of the offspring. Thus we have:—

No. of DD's in grandparentage.	Percentage of DD's in offspring.
4	100
3	67
2	41
1	22
0	11

In other words, the constitution of the grandparentage substantially modifies the offspring; there exists in this sense an "ancestral contribution" to the heritage.

Of course the gametic constitution RR follows precisely the same system of percentages, and is again influenced by ancestry.

If, however, we take the gametic constitution DR in such a population we find—

No. of DR's in grandparentage.	Percentage of DR's in offspring.
4	50
3	50
2	50
1	50
0	50

At first sight this seems to indicate that for this case there is no ancestral influence, where we should expect by increasing the number of DR's in the grandparentage to increase the number in the offspring. But this criticism is not valid, for, in the population we are dealing with, it is clear that DR is the modal or mean group, and that, accordingly, it is perfectly neutral in determining the regression or correlation of the *gametic* character. In other words, the deviations of the DR ancestry from the mean population gametic character are all zero and accordingly they have no weight in causing the offspring to deviate from the population norm. They have, in fact, no more effect on the offspring than, in the case of stature, a number of mediocre ancestors have in raising or lowering the average deviation of the offspring from the general population mean.

Lastly, turning from the gametic constitution to the somatic character, I have represented in the fourth and fifth columns of Table II the extent to which the dominant character is present in the ancestry, and in the accompanying table one sees the effect on the offspring:—

No. of grandparents with dominant character.	Percentage of offspring with dominant character.
4	89
3	78
2	59
1	33
0	0

It will thus be obvious that, judging solely by the patent, that is the somatic character of the grandparentage, there is a very marked influence of the ancestry on the heritage; that, if we select ancestry by somatic character only, we shall expect an influence on the offspring varying from 0 to 90 per cent. in intensity, according to the nature of the selection.

I think, therefore, that to deny the influence of ancestry—at any rate that influence in the sense in which the biometrician uses the term—is to deny the application of Mendelism to populations mating at random.

If we start with a population in which the proportions of DD's, DR's, and RR's are not those of a simple hybridisation, but given by

$$p\,(\mathrm{DD}) + 2q\,(\mathrm{DR}) + s\,(\mathrm{RR}),$$

then after the first generation of random mating the population will be

$$(p+2q+s)^2(p+q)^2(\mathrm{DD}) + 2\,(p+q)\,(s+q)\,(p+2q+s)^2(\mathrm{DR}) \\ + (p+2q+s)^2(s+q)^2(\mathrm{RR}),$$

or its constituents will be proportional to

$$(p+q)^2(\mathrm{DD})+2(p+q)(s+q)(\mathrm{DR})+(s+q)^2(\mathrm{RR}),$$

and this ratio is maintained ever afterwards.*

A little consideration will show that our Table II is obtained by a symbolic process which will not be affected if we replace D by $(p+q)$ D and R by $(s+q)$ R, so that to exhibit the results for a Mendelian population of any constituent proportions we have only to multiply all the numbers in any row of offspring of Table II by $(p+q)^2$ for a DD grandparent, by $(p+q)(s+q)$ for a DR grandparent, and by $(s+q)^2$ for an RR grandparent, starting with the stable population which arises after the first random mating. We then reach the following table for the case of classification by somatic characters, where for brevity I write: $p+q=\pi$, $s+q=\kappa$, and $\pi^2/\kappa^2=n=$ ratio of pure dominants to recessives in the stable population. It will be seen that whatever be the proportions of the Mendelian components in the original population, then a selection of grandparents influences widely the somatic characters of the offspring.

Whether, therefore, Mendelism be or be not the final word as to inheritance (and I personally, especially in the case of human characters, must continue to suspend my judgment), it is clear that ancestral influence cannot be denied in the case of any population mating at random and inheriting on Mendelian lines.

Table III.

No. of grandparents with dominant character.	Percentage of offspring with dominant character.
4	$100\dfrac{(n+1)(n+3)}{(n+2)^2}$
3	$100\dfrac{(n+1)(2n+5)}{2(n+2)^2}$
2	$100\dfrac{(5n+11)(n+1)}{6(n+2)^2}$
1	$100\dfrac{(n+1)}{2(n+2)}$
0	0

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* The stability after the first generation is very obvious, but, as far as I know, was first stated in print by G. H. Hardy, 'Science,' vol. 28, p. 49.

We have the following table for various values of n:—

No. of grandparents with dominant character.	Percentages of offspring with dominant character.						
	$n = 10.$	$= 4.$	$= 2.$	$= 1.$	$= \frac{1}{2}.$	$= \frac{1}{4}.$	$= \frac{1}{10}.$
4	99·3	97	94	89	84	80	77
3	95	90	84	78	72	68	65
2	78	72	66	59	54	50	48
1	46	42	37·5	33	30	28	26
0	0	0	0	0	0	0	0

When experimental work is adduced to demonstrate that ancestry has no influence, it will on investigation be found that the writer is:

(i) Confining his attention, as Mr. Darbishire, to isolated lines of inheritance, with restricted matings;

(ii) Asserting that a gametic knowledge of parents is equivalent to a gametic knowledge of ancestry.

In neither case does the argument touch the ancestral position, which is summed up in the assertions that if we measure inheritance by the resemblance of somatic characters between offspring and ancestry, then, in a population mating at random:

The more ancestors of any grade with a given somatic character the more offspring with that character.

For ancestry of different grades the influence is diminished in geometrical progression at each stage.

These principles were first deduced empirically from observations and records without any theory as to the mechanism of heredity. If Mendelism be true for any characters in cross-fertilised plants, then these principles hold also for heredity in that plant-population, for they are essential features of the Mendelian theory (and, as a matter of fact, of a good many other determinantal theories). No proof or disproof of them can be directly deduced from Mr. Darbishire's memoir, but since that memoir brings evidence for the truth of Mendelian theory, it indirectly asserts the truth that ancestry is influential, at least in the field where the biometrician expects and asserts it to play a part. This paper contains only another aspect of the results reached in 1904, but it provides in the simple case—the grandparentage—the actual percentage measures of the influence of ancestry according to Mendel. Its justification is the misinterpretation which is likely to be placed on the statement that "there is nothing like ancestral contributions within the limits of a single unit-character."*

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* Darbishire, 'Roy. Soc. Proc.,' B, vol. 81, p. 71.

On the Ancestral Gametic Correlations of a Mendelian Population mating at Random.

By Karl Pearson, F.R.S.

(Received April 2,—Read April 22, 1909.)

(1) The population to be considered in this paper is supposed to be initiated by a group of s_1 individuals with the protogenic constitution (AA), s_2 individuals with the allogenic constitution (aa), and s_3 individuals with the hybrid constitution (Aa), where the mating is given by the simple Mendelian formula: $(\mathrm{AA}) \times (aa) = 4\,(\mathrm{A}a)$. I do not assume at this stage any relation between the gametic constitution of an individual and its somatic character. I propose first to consider the correlation between any ancestor and the resulting array of offspring, when we regard only their gametic constitutions. I assume that all mating in the population is random, *i.e.* that every possible mating occurs simply in the proportions of the frequency of individuals of given gametic constitution in the population, and that there is no differential fertility or selective death-rate.

In a paper published in the 'Phil. Trans.,' vol. 203, A, 1904, p. 53 *et seq.*, I have dealt with the correlation between the *somatic* characters of the ancestry and the offspring in a population of a Mendelian character, more general in that I supposed the character to depend upon n couplets, and not a single Mendelian couplet, less general in that I supposed the population to have arisen from a series of initial hybridisations, and not from a mixture as in the present case of hybrids and members of two pure races in any proportions. In that paper I showed (a) that there was correlation between any ancestor and the offspring, (b) that the regression for any ancestor and the offspring was linear, and (c) that the correlations decreased in geometrical progression. These are the chief characteristics of the Law of Ancestral Heredity. It was clear that, judged by somatic characters only, ancestry was of importance. The result depended on Mendel's first principle of dominance being absolutely true. The values of the correlations were, however, less than those with which biometric work had made us familiar.

(2) In the present paper I start with a more general population, and investigate the correlation of the gametic not the somatic characters.

The general formula for the population before the first mating is

$$s_1\,(\mathrm{AA}) + 2s_3\,(\mathrm{A}a) + s_2\,(aa). \qquad \text{(i)}$$

After the first random mating it is

$$(s_1+s_3)^2\,(\mathrm{AA}) + 2\,(s_1+s_3)\,(s_2+s_3)\,(\mathrm{A}a) + (s_2+s_3)^2\,(aa).$$

I write this for brevity

$$p^2(\mathrm{AA})+2pq(\mathrm{A}a)+q^2(aa), \qquad \text{(ii)}$$

and this constitution remains permanent in all successive matings. Hence the standard deviations of the gametic constitutions remain the same generation after generation, and the correlation coefficient is in every case equal to the slope of the regression line. I shall determine the slope of this line which will give the correlation and show that the regression is truly linear in each case.

(3) I consider first the effect of individuals of each special type mating with the general population (ii).

(*a*) Type (AA): the array of offspring is $(p+q)[p(\mathrm{AA})+q(\mathrm{A}a)]$,
(*b*) Type (aa): ,, ,, $(p+q)[p(\mathrm{A}a)+q(aa)]$,
(*c*) Type (Aa): ,, ,, $\frac{1}{2}(p+q)[p(\mathrm{AA})+(p+q)(\mathrm{A}a)+q(aa)]$.

Thus, in seeking what any differentiated group

$$t_1(\mathrm{AA})+t_3(\mathrm{A}a)+t_2(aa)$$

produces when mated with the general population, *i.e.* when mated at random, all we have to do is to replace (AA), (Aa) and (aa) by the above three expressions respectively.

In this manner I obtained the array of offspring due to any parent, any grandparent and any great grandparent. These at once allowed me to reach the general law of distribution, and, assuming this, one multiplication by the general population (ii) demonstrated by induction the validity of the results reached. These are as follows:—

I term nth parent any individual n generations back in the direct ancestry: thus a 1st parent is the father or mother; a 2nd parent, a grandparent; a 3rd parent, a great grandparent, and so on.

(i) If the nth parent be an (AA), then the array of offspring due to random matings is

$$p^2(p+q)^{2(n-1)}(\tfrac{1}{2})^{n-1}\{(2^{n-1}p+q)p(\mathrm{AA})+[(2^n-1)p+q]q(\mathrm{A}a)$$
$$+(2^{n-1}-1)q^2(aa)\}.$$

(ii) If the nth parent be an (Aa), then the array of offspring is

$$(pq)(p+q)^{2(n-1)}(\tfrac{1}{2})^{n-1}\{[(2^n-1)p+q]p(\mathrm{AA})+[p^2+2(2^n-1)pq+q^2](\mathrm{A}a)$$
$$+[p+(2^n-1)q]q(aa)\}.$$

(iii) If the nth parent be an (aa), then the array of offspring is

$$q^2(p+q)^{2(n-1)}(\tfrac{1}{2})^{n-1}\{(2^{n-1}-1)p^2(\mathrm{AA})+[(2^n-1)q+p]p(\mathrm{A}a)$$
$$+(2^{n-1}q+p)q(aa)\}.$$

(4) These distributions correspond to the cases of 2, 1 and 0 A elements in the gametic constitution of the *n*th parent. And we have at once the following result:—

Number of protogenic elements in *n*th parent.	Average number of same elements in array of offspring.
2	$\dfrac{2^{n+1}p+2q}{2^n(p+q)} = \bar{y}_2,$
1	$\dfrac{(2^{n+1}-1)p+q}{2^n(p+q)} = \bar{y}_1,$
0	$\dfrac{(2^{n+1}-2)p}{2^n(p+q)} = \bar{y}_0.$

Accordingly, the average number of protogenic elements in the array of offspring decreases uniformly with the decrease in number of the like elements in the *n*th parent, *i.e.*

$$\bar{y}_2 - \bar{y}_1 = (\tfrac{1}{2})^n = \bar{y}_1 - \bar{y}_0.$$

Thus the regression between the *n*th parent and the offspring is linear, and the correlation coefficients form a geometrical series of ratio $\frac{1}{2}$, and first term $\frac{1}{2}$. Further, the exact constitution of the population, as far as the number of protogenic, allogenic or heterogenic individuals is concerned, is of no influence on the result at all. For all mixtures following the simple Mendelian rule: (AA) × (*aa*) = 4 (A*a*), the ancestral correlations for gametic constitution are:

Parental correlation	0·500
Grandparental correlation	0·250
Great grandparental correlation......	0·125 and so on.

It will be seen at once that these correlations are of the type ρ, ρ^2, ρ^3, etc., for which, in my memoir of 1896, I worked out the multiple regression formula, and showed that the ancestors were quite indifferent. "A knowledge of the ancestry beyond the parents in no way alters our judgment as to the size of organ or degree of characteristic probable in the offspring nor its variability."* This remark and the proof apply equally of course to gametic and to somatic characters if the correlation be of the above form.

(5) Accordingly there remains not the least antinomy between the Mendelian theory and the Law of Ancestral Heredity, if we confine our attention to gametic constitution. The Mendelian ancestry is correlated with the offspring in a series descending in a geometrical progression, and the regression is linear. The values of the correlation coefficients are

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* "Regression, Heredity, and Panmixia," 'Phil. Trans.,' A, vol. 187, 1896, p. 306.

precisely those which it was pointed out in 1896 would lead to a knowledge of the parental constitution* replacing that of the ancestry.

(6) The striking point, however, of the present investigation is that the values now shown theoretically to exist for the ancestral *gametic* correlations in a simple Mendelian mixture are very close to those determined for *somatic* characters in biometric investigations, whereas the *somatic* correlations for a Mendelian population, if we maintain intact the principle of absolute dominance, appear theoretically to be too low.

Thus the value for parental correlation in man, horse, dog and cattle is about 0·5, and for the grandparental correlation lies between 0·25 and 0·30; but this tendency in the grandparent to some slight excess on the Mendelian gametic value must not be given too much weight.

(7) It seems desirable to consider how far the results in my paper of 1904 for the somatic correlations are modified if we assume for our population

$$p^2(\mathrm{AA})+2pq(\mathrm{A}a)+q^2(aa),$$

and do not make $p = q$.

Assuming the principle of dominance to be absolute, I enquire what is the proportion of offspring possessing the dominant character† (*i.e.* (AA) or (Aa)) supposing the nth parent to possess it (*i.e.* to be (AA) or (Aa)); and again, what is the proportion possessing the dominant character, supposing the nth parent does not possess it (*i.e.* to be aa).

	Percentage of dominant offspring.
nth parent dominant in somatic character...	$100 \times \dfrac{2^{n-1}p(p+2q)^2+q^3}{2^{n-1}(p+q)^2(p+2q)}$,
nth parent recessive in somatic character ...	$100 \times \dfrac{2^{n-1}p(p+2q)^2-pq(p+2q)}{2^{n-1}(p+q)^2(p+2q)}$.

From this it follows that the correlation which is equal to the regression is

$$\frac{1}{2^{n-1}}\,\frac{q}{p+2q}.$$

If $p = q$, this is $\frac{1}{3}\,\frac{1}{2^{n-1}}$, in agreement with the conclusion of my memoir of 1904. But unless $q/(p+2q) = \frac{1}{2}$, *i.e.* the number of pure dominants in the population be vanishingly small (as well, of course, as the number of impure dominants!), this is not a series to which the form ρ, ρ^2, ρ^3 . . . applies, and when we judge (as we must in most instances in man) by the somatic and not the unknown gametic constitution, the ancestry does matter.

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* As a matter of fact, a knowledge of the gametic constitution of the ancestry in any generation would be equally sufficient with that of the parents.

† It is assumed that A is dominant over a.

The following table illustrates the percentages of dominant charactered offspring when we selected an ancestor of given character :—

Ancestor.	Percentage of dominants in offspring.					
	$p = 2q$.		$p = q$.		$q = 2p$.	
	Dominant.	Recessive.	Dominant.	Recessive.	Dominant.	Recessive.
Parent	91·7	66·7	83·3	50·0	57·8	33·3
Grandparent	90·3	77·8	79·2	62·5	56·7	44·4
3rd parent	89·6	83·3	77·1	68·7	56·1	50·0
4th parent	89·2	86·1	76·0	71·9	55·8	52·8
5th parent	89·1	87·5	75·5	73·4	55·7	54·2
6th parent	89·0	88·2	75·3	74·2	55·6	54·9
∞ th parent...............	88·9	88·9	75·0	75·0	55·6	55·6

It will be clear that the difference of the percentage of dominants in the offspring according as a parent, grandparent or great grandparent was dominant or recessive in somatic character is quite marked ; and only as we approach the higher ancestry, where the correlation is growing very weak, does the percentage difference grow imperceptible.

(8) That ancestry does not matter if we know the gametic constitution of the parents, that it does matter if we only know the somatic character of the parents appears to be the solution of one of the difficulties which some have found between the Mendelian and biometric methods of approaching the subject.

There is, however, I venture to think, another aspect of these results which is worthy of fuller consideration. Namely, the fairly close accordance now shown for the first time to exist between the ancestral gametic correlations in a Mendelian population and the observed ancestral somatic correlations suggests that the accordance between gametic and somatic constitutions is for at least certain characters possibly more intimate than is expressed by an absolute law of dominance. If (Aa) were a class, or possibly on a wider determinantal theory a group of several classes, marked by an individual somatic character—not invariably identical with the somatic character of (AA)—there would be little left of contradiction between biometric and Mendelian results as judged by populations sensibly mating at random. It is the unqualified assertion of the principle of dominance which appears at present as the stumbling block.

The Origin and Destiny of Cholesterol in the Animal Organism. Part V.—*On the Inhibitory Action of the Sera of Rabbits fed on Diets containing Varying Amounts of Cholesterol on the Hæmolysis of Blood by Saponin.*

By Mary T. Fraser and J. A. Gardner.

(Communicated by Dr. A. D. Waller, F.R.S. Received April 3,—Read May 6, 1909.)

(From the Physiological Laboratory, University of London, South Kensington.)

In an earlier paper* of this series it was shown that cholesterol is not excreted in the fæces of herbivorous animals, and that when rabbits are fed on a diet free from phytosterol but containing measured quantities of cholesterol, a portion of the latter substance is absorbed. The hypothesis was put forward that cholesterol is a substance which is strictly conserved in the animal economy; that when the destruction of the red blood corpuscles and possibly other cells takes place in the liver, their cholesterol is excreted in the bile, and that the cholesterol of the bile is reabsorbed in the intestine along with the bile salts, and finds its way into the blood stream to be used in cell-anabolism. It was also suggested that any waste of cholesterol might possibly be made up from that taken in with the food. In order to test this view, comparative estimations were made of the total cholesterol content of the blood of rabbits that had been respectively fed on bran which had previously been thoroughly extracted with ether, and on the same extracted bran with the addition of known amounts of cholesterol, care being taken that the animals were otherwise identically treated and were kept in good health. The results of the experiments showed that some, at any rate, of the cholesterol absorbed found its way into the blood stream. It seemed to us desirable to ascertain next whether the cholesterol was absorbed into the blood stream as such or in the form of esters or in both states, and also whether the phytosterol of vegetable food can be utilised for the formation of cholesterol in the organism. Owing to the small percentage of these substances in the blood, it did not seem probable that the chemical methods hitherto available for their estimation were sufficiently accurate to give reliable information unless in each experiment a larger number of animals was taken than we could conveniently attend to. It seemed likely, however, that a comparative study of the inhibitory effects of the sera of rabbits fed on different diets on the hæmolytic action of saponin on blood might throw light on the points mentioned.

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* "Origin and Destiny of Cholesterol," Part III, 'Roy. Soc. Proc.,' B, vol. 81, 1909 p. 109.

The experiments of Hausmann,[*] Abderhalden and Le Count[†] have proved that whereas cholesterol and phytosterol inhibit the action of saponin, their esters do not do so. More recently Windaus[‡] has shown that the inhibitory action of cholesterol and phytosterol is due to the fact that they form pharmacologically inactive compounds with saponin. We therefore decided to make a comparative study of the inhibitory action of the sera of rabbits fed respectively on ether-extracted bran and extracted bran with cholesterol, extracted bran and extracted bran with cholesterol esters, and, finally, extracted bran and extracted bran with phytosterol.

In the present paper an account is given of these experiments.

Method of Feeding the Animals under Experiment.—In each experiment, two large healthy rabbits, A and B, were fed for nine or ten days on bran which had been thoroughly extracted with ether. Excess of food was placed in the cages so that the animals could eat as much as they wished. In the case of rabbit A, after the third day a weighed amount of cholesterol mixed with a small quantity of moist extracted bran was given daily in addition, care being taken that the animal ate the whole of it. The rabbit was killed three or four hours after the last cholesterol meal, and the blood collected in a sterile vessel. The blood was allowed to clot and was placed in the refrigerator until the serum separated. The rabbit B was killed at the same time and its blood collected and treated in a similar manner. Great care was taken to keep the animals in good health and as far as possible under the same conditions. It is also desirable not to extend the experiment over too long a period lest the continued sameness of the diet should have a deleterious effect. If the animals are in bad health, more especially if they waste away and become emaciated, the results are entirely vitiated. This is well illustrated in the following experiment, which was commenced for another purpose and only continued as a matter of curiosity. Six medium-sized rabbits, weighing respectively 1·3, 1·4, 1, 1·2, 1·5, 2·3 kilogrammes, which were, to begin with, in very poor condition, were fed for three weeks on extracted bran. They continued in poor condition and became very emaciated, and had the experiment been further prolonged would no doubt have died. They yielded altogether only 190 grammes of blood. The blood was analysed by the method described in an earlier paper.[§] 0·1799 gramme of pure cholesterol benzoate was obtained, corresponding to 0·1423 gramme of cholesterol, or 0·0745 per cent. This is a higher percentage than we have ever found in healthy animals, even in the case of those fed on diets rich in cholesterol. We have not yet had an opportunity of making further experiments in this direction, but we do not think the result is in disagreement with the hypothesis referred to at the beginning of this paper.

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[*] 'Hofmeister's Beitr.,' vol. 6, p. 567.

[†] 'Ztschr. für exper. Path. und Ther.,' vol. 2, p. 199.

[‡] 'Ber. d. deut. chem. Ges.,' 1909, vol. 42, No. 1, p. 238.

[§] *Ibid.*

Method of Carrying out the Hæmolytic Experiments.—The method employed is to mix together a suspension of blood corpuscles, a solution of saponin and the serum, making up to a constant volume with physiological salt.

For this purpose the following solutions are used:—

(1) Physiological salt—a 0·85-per-cent. solution of NaCl (specially purified) in distilled water.

(2) Saponin (Merck)—a 0·01-per-cent. solution in the physiological salt.

(3) Rabbit's blood corpuscles—a 5-per-cent. suspension in physiological salt.

In carrying out the experiments, the mixtures of blood, saponin, serum, and physiological salt are very carefully measured into glass tubes with accurately ground glass stoppers. The tubes are then placed in clamps fitted on to a circular plate of wood in such a manner that they radiate from the centre to the circumference. This disc, with the tubes attached, is slowly revolved in a vertical plane by means of a clockwork drum, the whole apparatus being kept at a constant temperature of 37° by placing it in an incubator.

As the tubes are completely inverted during the revolution of the disc, the corpuscles are kept equally distributed throughout the mixture without violently agitating the contents.

Elastic bands hold the stoppers securely in position to prevent their coming out of the tubes as they expand with the heat of incubation. The most favourable period of incubation was found to be three hours. After this the tubes are placed on ice and the corpuscles allowed to settle, or they may be at once centrifugalised. In either case the amount of hæmolysis is judged from the colour of the clear supernatant liquid.

By taking readings of a tintometer [Michael], on consecutive days, it was found that the tint of the tubes kept on ice did not vary, this showing that further hæmolysis than that taking place during incubation was prevented entirely by placing on ice. The ice method was adopted generally.

Experiments to ascertain whether Cholesterol administered with the Food and absorbed by the Animal appears in the Blood Stream as such.

Experiment I.—It was first necessary to ascertain the least amount of saponin solution which would produce complete hæmolysis in a given volume of a 5-per-cent. suspension of rabbit's blood corpuscles. The results of the experiment are embodied in the following table :—

Table I.

Amount of blood, 5-per-cent. suspension.	Amount of saponin, 0·001 per cent.	Amount of NaCl, 0·85 per cent.	Result.
c.c.	c.c.	c.c.	
2	0·1	3·9	Incomplete hæmolysis
2	0·25	3·75	,, ,,
2	0·5	3·5	,, ,,
	0·01-per-cent. sol.		
2	0·1	3·9	,, ,,
2	0·25	3·75	,, ,,
2	0·5	3·5	,, ,,
2	1	3	Complete hæmolysis
2	2	2	,, ,,

Incubated for three hours at 37° C.

Thus 1 c.c. of a 0·01-per-cent. solution of saponin in a 0·85-per-cent. solution of NaCl completely hæmolyses 2 c.c. of a 5-per-cent. blood suspension in a total volume of 6 c.c.

It was found, however, in the course of the experiments, that the least amount of saponin needed to cause hæmolysis varied slightly with different specimens of rabbit's blood. For this reason it was necessary always to have the serum of a rabbit fed on extracted bran alone to compare with the serum of a rabbit fed on extracted bran and cholesterol, etc., so that the comparative experiments were carried out on the corpuscles of the same rabbit.

Experiment II.—Rabbit 1; fed on extracted bran for 10 days; weight of rabbit without blood = 1·7 kilogrammes.

Rabbit 2; fed for 10 days on extracted bran, during the last six days given $\frac{1}{4}$ gramme cholesterol per day. Weight of rabbit without blood = 2·5 kilogrammes.

Rabbit 3; fed for 10 days on extracted bran, during the last six days given $\frac{1}{4}$ gramme cholesterol per day for the first three days, 1 gramme per day for the last three days. Total, $3\frac{3}{4}$ grammes cholesterol.

Animals kept under the same conditions and killed at the same time. Sera collected 36 hours after death.

Results of experiment are given in the following tables :—

Table II.—Inhibitory Action of Serum of Extracted Bran-fed Rabbit (Rabbit 1).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Almost complete hæmolysis
2	0·05	2·95	1	Slight hæmolysis
2	0·1	2·9	1	Trace hæmolysis
2	0·5	2·5	1	No hæmolysis
2	1	2	1	,,

Table III.—Inhibitory Action of Serum of Rabbit fed on Extracted Bran + Cholesterol (Rabbit 2).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	No hæmolysis
2	0·05	2·95	1	,,
2	0·1	2·9	1	,,
2	0·5	2·5	1	,,
2	1	2	1	,,

Table IV.—Inhibitory Effect of Serum of Rabbit fed on Extracted Bran + Cholesterol (Rabbit 3).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	No hæmolysis
2	0·05	2·95	1	,,
2	0·1	2·9	1	,,
2	0·5	2·5	1	,,
2	1	2	1	,,

Incubated for three hours at 37° C.

In order to control these figures, the experiments were repeated the following day, with identical results. It was found, however, that when kept over a period of several days the sera lost their inhibitory effect to some extent, but even then the relative strengths remain in the same proportion.

Experiment III.—Rabbit 4; fed on extracted bran for 14 days, weight = 1·5 kilogrammes.

Rabbit 5; fed on extracted bran for 14 days, the last eight days with cholesterol in addition; $\frac{1}{4}$ gramme the two first, $\frac{1}{2}$ gramme the next four, and 1 gramme the last two days. Total cholesterol, $4\frac{1}{2}$ grammes; weight = 2 kilogrammes.

Animals kept under similar conditions and killed at the same time. Serum collected day after death.

The results of experiments are tabulated below:—

Table V.—Inhibitory Effect of Serum of Rabbit fed on Extracted Bran (Rabbit 4).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Almost complete hæmolysis
2	0·05	2·95	1	Considerable hæmolysis
2	0·1	2·9	1	A trace hæmolysis
2	0·5	2·5	1	No hæmolysis

Table VI.—Inhibitory Effect of Serum of Rabbit fed on Extracted Bran + Cholesterol (Rabbit 5).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Considerable hæmolysis
2	0·05	2·95	1	A trace hæmolysis
2	0·1	2·9	1	No hæmolysis
2	0·5	2·5	1	”

Exactly similar results were given by the sera heated for over an hour to 56° C., as the following table shows. The heating was performed with the object of showing that the inhibitory action of the sera on hæmolysis is not due to an organic enzyme.

Table VII.—Inhibitory Action of Heated Serum of Rabbit fed on Extracted Bran alone (Rabbit 4).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Almost complete hæmolysis
2	0·05	2·95	1	Hæmolysis
2	0·1	2·9	1	Slight hæmolysis
2	0·5	2·5	1	No hæmolysis

Table VIII.—Inhibitory Action of Heated Serum of Rabbit fed on Extracted Bran + Cholesterol (Rabbit 5).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Hæmolysed
2	0·05	2·95	1	A trace hæmolysis
2	0·1	2·9	1	No hæmoylsis
2	0·5	2·5	1	,,

A third series of observations was made the same day, with the sera in a 10-per-cent. solution of physiological salt. The results are given below:—

Table IX.—Inhibitory Action of 10-per-cent. Solution of Serum of Rabbit fed on Extracted Bran (Rabbit 4).

Amount of blood, 5-per-cent. suspension.	Amount of serum, 10 per cent.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·25	2·75	1	Almost complete hæmolysis
2	0·5	2·5	1	Hæmolysis
2	1·0	2·0	1	Slight hæmolysis
2	1·5	1·5	1	No hæmolysis

Table X.—Inhibitory Action of 10-per-cent. Solution of Serum of Rabbit fed on Extracted Bran + Cholesterol (Rabbit 5).

Amount of blood, 5-per-cent. suspension.	Amount of serum, 10 per cent.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·25	2·75	1	Hæmolysed
2	0·5	2·5	1	A trace hæmolysis
2	1·0	2·0	1	No hæmolysis
2	1·5	1·5	1	,,

We repeated these experiments with three other pairs of rabbits, and the results were so similar that we need not recapitulate them. It is obvious, therefore, that the cholesterol absorbed from the food appeared in the blood stream in the free state. Whether any entered in the form of esters it is impossible to say, but experiments are in progress which we hope will throw light on this subject.

It now seemed of interest to ascertain whether, the animals being fed on esters of cholesterol in addition to extracted bran, the esters were absorbed entirely as such, or whether they undergo hydrolysis in the process of digestion, and find their way into the blood stream as free cholesterol, in part, at any rate.

For this purpose we made use of pure cholesterol oleate and cholesterol stearate for feeding the animals.

Experiment IV.—Rabbit 6; fed on extracted bran for 18 days; weighed 2·2 kilogrammes at beginning, 2·3 kilogrammes at end of experiment.

Rabbit 7; fed on extracted bran for eight days, the last six days fed with cholesterol oleate in addition; $\frac{1}{4}$ gramme for four days, 1 gramme per day for two days; weight of animal = 2 kilogrammes at beginning and end of feeding.

Rabbit 8; fed on extracted bran for eight days, the last six days fed with cholesterol stearate in addition; $\frac{1}{4}$ gramme for four days, 1 gramme per day for two days; weight of animal = 2 kilogrammes at beginning and end of feeding.

Animals were kept under the same conditions and killed at the same time. Serum collected next day.

Results of experiment are tabulated below:—

Table XI.—Inhibitory Action of Serum of Rabbit fed on Extracted Bran (Rabbit 6).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·725	1·25	Almost complete hæmolysis
2	0·05	2·7	1·25	Considerable hæmolysis
2	0·1	2·65	1·25	„ „
2	0·25	2·5	1·25	Slight hæmolysis

Table XII.—Inhibitory Action of Serum of Rabbit fed on Cholesterol Oleate in addition to Extracted Bran (Rabbit 7).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·725	1·25	Hæmolysis
2	0·05	2·7	1·25	„
2	0·1	2·65	1·25	Slight hæmolysis
2	0·25	2·5	1·25	No hæmolysis

Table XIII.—Inhibitory Action of Serum of Rabbit fed on Cholesterol Stearate in addition to Extracted Bran (Rabbit 8).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·725	1·25	Hæmolysis
2	0·05	2·7	1·25	„
2	0·1	2·65	1·25	Slight hæmolysis
2	0·25	2·5	1·25	No hæmolysis

The experiments were again performed with the sera after heating them to 56° C. with the same object as in the case of the previous experiments. The following tables give the results:—

Table XIV.—Inhibitory Action of Serum of Rabbit fed on Extracted Bran (Heated) (Rabbit 6).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·725	1·25	Almost complete hæmolysis
2	0·05	2·7	1·25	Considerable hæmolysis
2	0·1	2·65	1·25	" "
2	0·25	2 5	1·25	Slight hæmolysis

Table XV.—Inhibitory Action of Heated Serum of Rabbit fed on Extracted Bran + Cholesterol Oleate (Rabbit 7).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·725	1·25	Hæmolysis
2	0·05	2·7	1·25	"
2	0·1	2·65	1·25	Slight hæmolysis
2	0·25	2·5	1·25	No hæmolysis

Table XVI.—Inhibitory Action of Heated Serum of Rabbit fed on Extracted Bran + Cholesterol Stearate (Rabbit 8).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·725	1·25	Hæmolysis
2	0·05	2·7	1·25	"
2	0·1	2·65	1·25	Slight hæmolysis
2	0·25	2·5	1·25	No hæmolysis

They were also carried out with 10-per-cent. solution of the sera in physiological salt.

Table XVII.—Inhibitory Action of Serum in 10-per-cent. Solution. Extracted Bran-fed Rabbit (Rabbit 6).

Amount of blood, 5-per-cent. suspension.	Amount of serum, 10 per cent.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·25	2·5	1·25	Almost complete hæmolysis
2	0·5	2·25	1·25	Considerable hæmolysis
2	1·0	1·75	1·25	" "
2	2·5	0·25	1·25	Slight hæmolysis

Table XVIII.—Inhibitory Action of Serum of Rabbit fed on Extracted Bran + Cholesterol Oleate in 10-per-cent. Solution (Rabbit 7).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·25	2·5	1·25	Hæmolysis
2	0·5	2·25	1·25	"
2	1·0	1·75	1·25	Slight hæmolysis
2	2·5	0·25	1·25	No hæmolysis

Table XIX.—Inhibitory Action of Serum of Rabbit fed on Extracted Bran + Cholesterol Stearate in 10-per-cent. Solution (Rabbit 8).

Amount of blood, 5-per-cent. suspension.	Amount of serum, 10 per cent.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·25	2·5	1·25	Hæmolysis
2	0·5	2·25	1·25	"
2	1·0	1·75	1·25	Slight hæmolysis
2	2·5	0·25	1·25	No hæmolysis

These experiments clearly show that the esters of cholesterol undergo hydrolysis during the digestive process and appear, partially at any rate, in the blood stream as free cholesterol.

As cholesterol is not a normal constituent of the food of rabbits, it appeared interesting to find out (1) whether vegetable phytosterol is absorbed by the animal if given in the food; (2) whether this can be utilised by the animal.

Experiment V.—To ascertain whether the phytosterol of the food in the case of rabbits is excreted unchanged or whether any is absorbed or destroyed during digestion.

A large healthy rabbit was fed for eight days on a mixture of equal parts of extracted bran and extracted wheat germ, and the fæces were collected during the last seven days. The fæces, which when dried weighed 60 grammes, were extracted with ether and the ethereal solution saponified with sodium ethylate. The unsaponifiable matter was obtained in the form of a clear stiff oil, weighing 0·4115 gramme. This was dissolved in 5 c.c. absolute alcohol and left to crystallise. A small quantity of greasy crystalline matter separated, too small to be readily purified. It was not cholesterol, but consisted of the crystalline "phytosterol" of bran, from which it is difficult to free completely the original bran by simple extraction.

The animal was then fed for one day on the same diet, the fæces being discarded, and for the following eight days on a daily ration of $\frac{3}{4}$ gramme of phytosterol (from wheat-germ), 30 to 40 grammes of extracted bran and an equal quantity of extracted germs of wheat, care being taken that the animal ate the whole of the phytosterol—2 grammes in all. It was then fed for four more days on the same diet, but without phytosterol. The fæces collected during the 12 days weighed, after drying, 189 grammes. The animal remained in good health and its weight was constant all through the experiment. The fæces were treated as before and yielded 2·3965 grammes of greasy unsaponifiable matter. This was repeatedly recrystallised from alcohol and two crops of pure phytosterol were obtained, the weights and melting points of which were: crop 1, 0·6465 gramme, M.P. = 132° C.; crop 2, 0·603 gramme, M.P. = 132° C. The mother liquors and residues were then evaporated to dryness and heated for a few minutes at a temperature of 180°—200° C. with benzoyl chloride. The product was then poured into a suitable quantity of alcohol and allowed to stand. The difficultly soluble crystalline matter which separated was recrystallised from hot alcohol. In this manner 0·149 of white phytosterol benzoate was obtained which on recrystallisation from ethyl acetate was obtained in characteristic crystalline form and melted at 141° C. 1·3675 grammes of pure phytosterol were therefore recovered out of the 2 grammes administered.

A consideration of the quantity of phytosterol recovered and also of the relative quantities of unsaponifiable matter obtained in the two parts of the experiment makes it clear that some of the phytosterol of the food was either destroyed or absorbed, but most probably absorbed.

Experiment VI.—In order to ascertain whether phytosterol given in the food has the same effect as cholesterol.

For this purpose animals were fed with actual bran and wheat-germ, which is rich in phytosterol, and other animals were fed on extracted bran with the addition of measured quantities of pure phytosterol.

Rabbit 9; fed on extracted bran for 10 days; weight of animal = 1·4 kilogrammes.

Rabbit 10; fed on extracted bran for three days, then ordinary bran and wheat-germ for six days; weight of animal = 1·7 kilogrammes. The following table gives the result of experiment:—

Table XX.—Inhibitory Effect of Serum of Rabbit fed on Extracted Bran (Rabbit 9).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Almost complete hæmolysis
2	0·05	2·95	1	Slight hæmolysis
2	0·1	2·9	1	A trace hæmolysis
2	0·5	2·5	1	No hæmolysis

Table XXI.—Inhibitory Effect of Serum of Rabbit fed on Ordinary Bran and Wheat-germ (Rabbit 10).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Hæmolysis
2	0·05	2·95	1	Slight hæmolysis
2	0·1	2·9	1	No hæmolysis
2	0·5	2·5	1	„

This experiment was repeated the following day with identical results.

Rabbit 11; fed on extracted bran for 11 days; weight of animal = 2 kilogrammes.

Rabbit 12; fed on ordinary bran and wheat-germ for nine days, having been fed with extracted bran for two days previously; weight of animal = 2 kilogrammes.

The animals were kept under exactly similar conditions and killed at the same time. Serum collected day after death. The results are tabulated below:—

Table XXII.—Inhibitory Action of Serum of Rabbit fed on Extracted Bran (Rabbit 11).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Almost complete hæmolysis
2	0·05	2·95	1	Hæmolysis
2	0·1	2·9	1	Slight hæmolysis
2	0·5	2·5	1	No hæmolysis

Table XXIII.—Inhibitory Action of Serum of Rabbit fed on Ordinary Bran and Wheat-germ (Rabbit 12).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Hæmolysis
2	0·05	2·95	1	Slight hæmolysis
2	0·1	2·9	1	A trace hæmolysis
2	0·5	2·5	1	No hæmolysis

Experiments were also carried out with the sera after heating for over an hour to 55° C., and in 10-per-cent. solutions made up with physiological salt. The results agreed entirely with the above tables.

The serum of the rabbits fed on ordinary bran and wheat-germ shows a slightly greater inhibitory power than the serum of the rabbit fed on extracted bran. This seems to indicate (1) that some of the phytosterol of the wheat-germ found its way into the blood stream, or (2) possibly caused an increase of cholesterol in the blood. Rabbits experimented on disliked the wheat-germ so that it was often neglected, the extracted bran being always given the preference. We therefore resolved to give, in subsequent experiments, weighed quantities of pure phytosterol.

Rabbit 13; fed on extracted bran for seven days.

Rabbit 14; fed on extracted bran for 12 days with phytosterol in addition, eight days with $\frac{3}{4}$ gramme per day, then four days with $\frac{1}{2}$ gramme per day.

Animals kept under similar conditions.

Table XXIV.—Inhibitory Action of Serum of Rabbit fed with Extracted Bran (Rabbit 13).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Complete hæmolysis
2	0·05	2·95	1	Considerable hæmolysis
2	0·1	2·9	1	Slight hæmolysis
2	0·5	2·5	1	No hæmolysis

Table XXV.—Inhibitory Action of Serum of Rabbit fed with Extracted Bran + Phytosterol (Rabbit 14).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Hæmolysis not complete
2	0·05	2·95	1	A trace hæmolysis
2	0·1	2·9	1	No hæmolysis
2	0·5	2·5	1	"

These experiments were repeated with the same sera, on consecutive days, three times altogether, and in every case the result was identical.

Further, it was found that on addition of greater quantities of saponin to the same volume of blood, if the serum were added in proportional quantities, hæmolysis was in every case prevented, if the ratio between saponin and serum were the same as that which prevented hæmolysis in the above experiment.

The table is given below:—

Table XXVI.—Inhibitory Effect of Serum of Rabbit fed on Extracted Bran with Phytosterol in addition (Rabbit 14).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Hæmolysis
2	0·05	2·95	1	A trace hæmolysis
2	0·1	2·9	1	No hæmolysis
2	0·15	2·35	1·5	"
2	0·2	1·8	2	"
2	0·25	1·25	2·5	"

This is interesting as giving an indication of the quantitative reaction between the serum and saponin, and therefore of its chemical nature.

In order to ascertain whether the result would be affected by heating or dilution, however, another pair of rabbits was used.

Rabbit 15; fed for 14 days on extracted bran. Weight of animal = 1·5 kilogrammes.

Rabbit 16; fed on ordinary bran and wheat-germ for 13 days, the last eight days with phytosterol in addition; for six days ¼ gramme per day, then ¾ gramme the next day, and 1 gramme the last day.

Animals kept under similar conditions and killed at the same time. The results of experiments are given below:—

Table XXVII.—Inhibitory Effect of Serum of Rabbit fed on Extracted Bran (Rabbit 15).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Hæmolysis not quite complete
2	0·05	2·95	1	Considerable hæmolysis
2	0·1	2·9	1	Slight hæmolysis
2	0·5	2·5	1	No hæmolysis

Table XXVIII.—Inhibitory Effect of Serum of Rabbit fed with Phytosterol in addition to Ordinary Bran and Wheat-germ (Rabbit 16).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Hæmolysed
2	0·05	2·95	1	A trace of hæmolysis
2	0·1	2·9	1	No hæmolysis
2	0·5	2·5	1	„

Table XXIX.—Inhibitory Effect of Rabbit fed on Extracted Bran heated to 56° C. for an hour (Rabbit 15).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Hæmolysed (not quite complete)
2	0·05	2·95	1	Considerable hæmolysis
2	0·1	2·9	1	Slight hæmolysis
2	0·5	2·5	1	No hæmolysis

Table XXX.—Inhibitory Effect of Serum of Rabbit fed on Phytosterol in addition to Ordinary Bran and Wheat-germ. Heated to 56° C. for an hour (Rabbit 16).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·025	2·975	1	Hæmolysed
2	0·05	2·95	1	A trace hæmolysis
2	0·1	2·9	1	No hæmolysis
2	0·5	2·5	1	"

Table XXXI.—Inhibitory Effect of Serum of Rabbit fed on Extracted Bran in 10-per-cent. Solution made up with Physiological Salt (Rabbit 15).

Amount of blood, 5-per-cent. suspension.	Amount of serum, 10 per cent.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·25	2·75	1	Hæmolysis almost complete
2	0·5	2·5	1	Considerable hæmolysis
2	1·0	2	1	Slight hæmolysis
2	1·5	1·5	1	No hæmolysis

Table XXXII.—Inhibitory Effect of Serum of Rabbit fed on Phytosterol in addition to Ordinary Bran and Wheat-germ in 10-per-cent. Solution of Physiological Salt (Rabbit 16).

Amount of blood, 5-per-cent. suspension.	Amount of serum.	Amount of NaCl, 0·85 per cent.	Amount of saponin, 0·01 per cent.	Result.
c.c.	c.c.	c.c.	c.c.	
2	0·25	2·75	1	Hæmolysed
2	0·5	2·5	1	A trace hæmolysis
2	1	2	1	No hæmolysis
2	1·5	1·5	1	„

These experiments fully confirm the conclusion arrived at from a comparison of the sera fed on extracted bran and on ordinary bran + wheat-germ.

Conclusions.

1. When cholesterol is given with the food of rabbits, some is absorbed and finds its way into the blood stream as free cholesterol, only a portion of the total cholesterol given in the food is absorbed, the rest being excreted unchanged. The amount of cholesterol which finds its way into the blood stream was not increased in our experiments by increasing the amount given in the food. It would appear probable, therefore, that the animals only take up such an amount of cholesterol as they can utilise.

2. Cholesterol when in the form of esters undergoes hydrolysis in part, at any rate, during digestion, and appears in the blood stream as free cholesterol.

3. When animals are fed on phytosterol, this substance is in part absorbed, just as in the case of cholesterol, and appears in the blood stream either itself or in the form of cholesterol. The latter point can, however, only be decided by the examination of very large quantities of the blood of animals fed on phytosterol.

Experiments are now in progress which we hope will decide this question.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work.

The Influence of Glucosides on the Growth of Acid-fast Bacilli, with a New Method of Isolating Human Tubercle Bacilli directly from Tuberculous Material contaminated with other Micro-organisms. (Preliminary Note.)

By F. W. Twort.

(Communicated by Leonard Hill, F.R.S. Received February 18,—Read March 4, 1909.)

This investigation was undertaken to test the action of acid-fast bacilli on the glucosides, and to see how far any fermentation reactions obtained would differ with the various strains of human and bovine tubercle bacilli tested, and also to obtain if possible a better medium on which to isolate and grow tubercle bacilli. In all, 43 glucosides were tested with acid-fast bacilli, including human and bovine tubercle bacilli, but there was no evidence of fermentation with any of the glucosides.

One glucoside, *ericolin,* was found to kill off a large number of species of micro-organisms, especially bacilli of the colon group and various cocci, but had very little effect on the acid-fast group of bacilli.

By means of this glucoside the isolation of tubercle bacilli directly from human sputum contaminated with other organisms becomes quite easy. The glucoside should be made up with distilled water in a 2-per-cent. solution; a lump of sputum is then placed into a test-tube containing the ericolin and placed at 38° C. for $\frac{3}{4}$ hour to 1 hour; sub-cultures are then made on to Dorset's egg medium, and pure growths of tubercle bacilli will be obtained in 14—28 days; the tubes are sometimes contaminated with a few other organisms, chiefly tiny colonies of streptococci and slow-growing colonies of organisms of the streptothrix group, but they are so few that they in no way interfere with the tubercle colonies, which can be easily sub-cultured.

Reciprocal Innervation of Antagonistic Muscles. Fourteenth Note.—On Double Reciprocal Innervation.

By C. S. Sherrington, D.Sc., F.R.S.

(Received March 25,—Read May 6, 1909.)

(From the Physiology Laboratory, University of Liverpool.)

I.—Reflex excitation and inhibition when brought to play simultaneously on the motoneurones of an extensor muscle can be so balanced that there results in the muscle a contraction, the degree of which evidences algebraic summation of the two opposed influences, the inhibitory and the excitatory.* This summation is obtainable not only in the decerebrate animal but also in the purely spinal (fig. 1). In the latter it proves obtainable in preparations quite recently made spinal, for instance, after decapitation. This circumstance much facilitates the physiological study of the phenomenon. In other words, the grading (fig. 1) of reflex contraction of the extensor by varying intensity of inhibition acting along with reflex excitation can be studied in the animal freshly made spinal as well as in the animal in the decerebrate condition. The muscle which I have chiefly employed in the purely spinal preparation is the isolated extensor of the knee in the decapitated cat.

A difference does indeed exist between the reactions of the preparation in the decapitated and in the decerebrate condition. In the decapitated preparation, as in the decerebrate, the reflex effect of any inhibitory afferent is easily seen if stimulation of that afferent is employed concurrently with stimulation of an excitatory afferent. If however the inhibitory afferent is stimulated during the ordinary resting condition of the preparation, there is usually no change in the muscle to show that the inhibitory afferent is producing any effect at all (fig. 2). This is because the extensor muscle in the decapitated preparation is not exhibiting tonus and lies relaxed, therefore affording no background of contraction against which an inhibitory reflex can reveal itself by causing relaxation. Even in this condition it can, however, easily be shown that the inhibitory afferent is then really producing a state of inhibition in the preparation, although that state is not made evident by any further relaxation. If a stimulus sufficient to cause reflex contraction of the muscle be applied to the excitatory afferent while the inhibitory afferent, although apparently without effect, is being stimulated, the excitatory afferent is found to be ineffective (or only partially effective) then; but it immediately becomes effective (or more effective) on withdrawal of the concurrent inhibitory stimulation (fig. 2).

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* Sherrington, 'Roy. Soc. Proc.,' Note XIII, Nov., 1908.

Similarly with flexor muscles. The flexors of the knee can be suitably studied with *semitendinosus* or *biceps femoris.* With the latter, the posterior part only of the muscle need be used, the anterior part not being a true knee flexor (cat, dog). The muscle, *e.g.*, semitendinosus, is isolated by detachment from tibia and by severance in both hind limbs of all nerves other than its own. The reflex contraction or relaxation of the muscle can then be studied by the myograph. Reflex contraction is easily obtained by stimulation

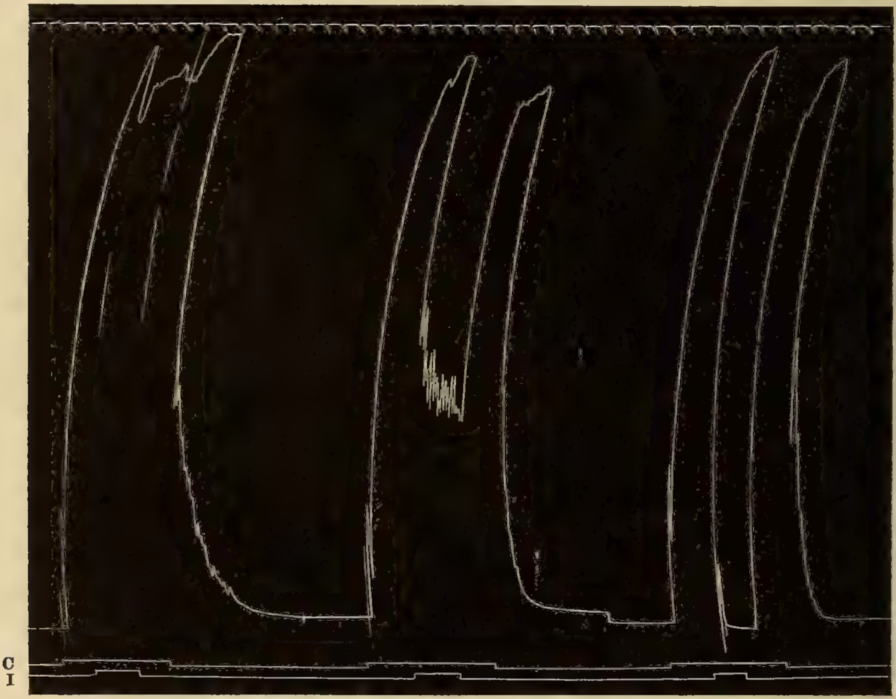

Fig. 1.—Reflex contraction of Vastocrureus in spinal mammal (decapitated cat), excited by faradic stimulation of contralateral popliteal nerve (C). During this contraction the ipselateral peroneal nerve was faradised for the time shown by the lower signal (I), and produced a lessening of the reflex contraction; this inhibitory effect is greater according as the intensity of the stimulation of the ipselateral nerve is increased. In the second observation it reduces the reflex contraction to less than half; in the third observation it suppresses it altogether for so long as the inhibitory stimulus endures. In the third observation, with the strong inhibition, so abrupt and quick is the suppression of the contraction that the writing lever gives a vibratory shake at the end of its fall. The tremor seen to occur in the second observation during the period of concurrent excitation and inhibition is usual, and is more marked in the decapitated preparation than in the decerebrate. Time in seconds above.

(mechanical or electrical) of the central stump of almost any afferent nerve of the ipselateral limb. If while this reflex contraction is in progress the central stump of a nerve, *e.g.*, popliteal, of the contralateral fellow limb be suitably faradised, there ensues immediate relaxation of the contracting semitendinosus (fig. 3). But if the contralateral afferent be stimulated while the preparation is at rest there may be no evidence of inhibition or of any other effect. The reflex stimulus might then be supposed not to be exerting any influence whatever on the flexor muscle. That is because there is then in the muscle no tonic or other contraction against which the reflex inhibition can show. That the reflex inhibitory influence is, however, really at work

FIG. 2.

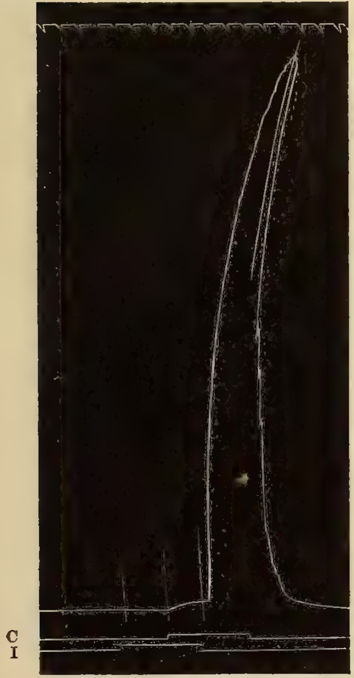

FIG. 3.

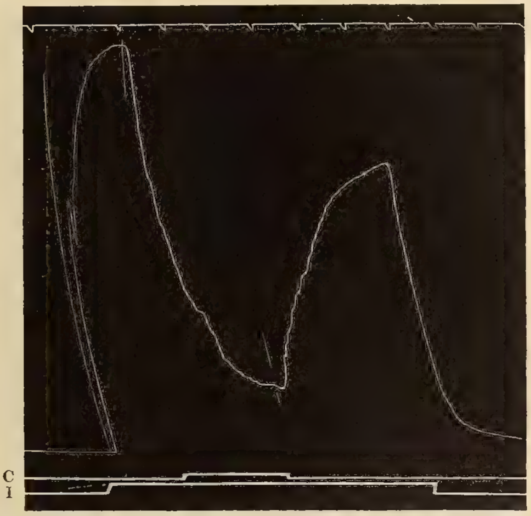

FIG. 2.—Reflex contraction of Vastocrureus muscle evoked by stimulation of central end of contralateral peroneal nerve (C). Before this stimulation a stimulation of the ipselateral peroneal (I) was commenced. This latter stimulation produced no visible effect on the muscle, but it is only on its cessation that the contralateral stimulation is able to produce a contraction. Cat, decapitated preparation. Time in seconds above.

FIG. 3.—Reflex contraction of Semitendinosus, a knee-flexor, evoked by stimulation of central end of ipselateral peroneal nerve (I), and inhibited during concurrent stimulation of contralateral popliteal nerve (C). Cat, decapitated preparation. Time in seconds above.

can be shown by the same device as that employed above to reveal it under similar circumstances in the extensor muscle.

Another way in which the hidden inhibition can be revealed is by administering a small dose of strychnine. It was pointed out previously that strychnine converts reflex inhibition of the extensor and certain other muscles into reflex excitation. I find this hold good also in regard to reflex inhibition of flexors. Intravenous injection of 0·15 milligramme strych. hydrochl. per kilogramme (cat) almost at once converts the inhibitory effect of a contralateral afferent on semitendinosus into an excitatory effect (fig. 4).

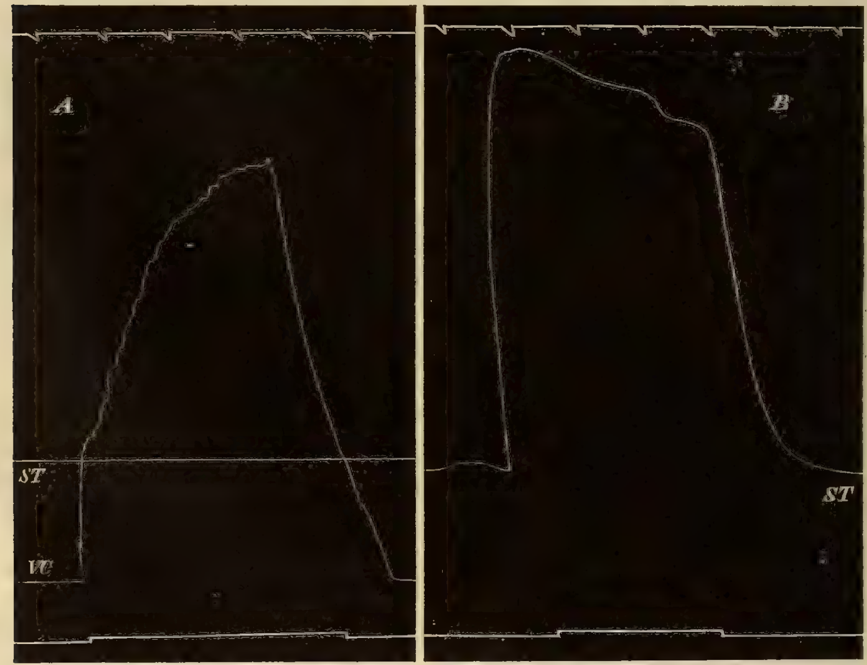

Fig. 4.—Effect of stimulation of contralateral peroneal on Semitendinosus; A, before strychnine; B, after. In B the contraction of vastocrureus is not included, but semitendinosus (ST), which had shown no contraction in A, because the stimulus was inhibitory to it, in B under the same stimulus exhibits the marked contraction shown, the small dose of strychnine having converted reflex inhibition into reflex excitation. The contraction long outlasts the period of stimulation in B.

Stimulation of the afferent then, in the resting preparation, instead of leaving the muscle apparently untouched, produces reflex contraction of it. The influence on the flexor motoneurones, which was previously unable to show itself in the relaxed muscle, because inhibitory, at once reveals its existence, because under strychnine it has become an excitatory influence.

And this holds good with the flexor muscle both in the decapitated and in the decerebrate preparation. The view that strychnine, by lessening an intracentral resistance, so allows the nervous impulse access where it previously had none, is therefore hardly applicable to the flexor any more than to the extensor: in both cases the effect is well explained by the strychnine converting reflex inhibition into reflex excitation.

II.—The algebraic summation of reflex excitatory and inhibitory effect shows itself in the flexor muscle as well as in the extensor. Suppose reflex contraction of semitendinosus to be in progress under faradisation of the central stump of an afferent nerve, *e.g.*, peroneal, of the ipselateral limb. If then the central stump of an afferent nerve, *e.g.*, popliteal, of the contralateral fellow limb be suitably faradised, there ensues an inhibitory relaxation of the contracting muscle. When the relative intensities of the stimulations of the two nerves are appropriately adjusted, the contraction of the muscle is not wholly abolished but is merely lessened in degree. The inhibitory stimulus under these circumstances causes first a rapid partial lengthening of the muscle, indicated by a descent of the myogram line; this rapid descent reduces the contraction to a certain level of diminished contraction, which is then equably maintained during the further continuance of the inhibitory stimulus, giving a plateau in the myogram. If, then, the inhibitory stimulus be withdrawn, the excitatory still continuing as before, the reflex contraction increases again forthwith, regains a higher level, and, subject to fatigue, continues until finally the excitatory stimulus is itself withdrawn. That this grading which involves inhibition as one factor can be accomplished in either of two ways is proved by observation as follows:—Throughout a (fig. 5) consecutive series of reflexes the excitatory stimulus can be kept of constant intensity, while the inhibitory is varied in intensity in the sequent reflexes. When this is done, the amount which inhibition can subtract from the reflex contraction is found to be graduable from the hardly perceptible up to complete cancelling of all contraction for the time being. Conversely, a series of reflexes (fig. 6), in which the inhibitory stimulus remains constant in intensity but the excitatory is varied from reflex to reflex, produces degrees of reflex contraction extending from hardly perceptible lessening of the reflex contraction even to complete relaxation of the contracted muscle.

With flexor motoneurones, therefore, as with extensor, a grading of reflex contraction is obtainable by employing concurrently with reflex excitation an appropriate reflex inhibition. The algebraic summation of these two opposed influences results in an infinite series of grades of intensity of contraction both in the extensor and in the flexor muscle.

III.—The records show that the combining of any particular value of

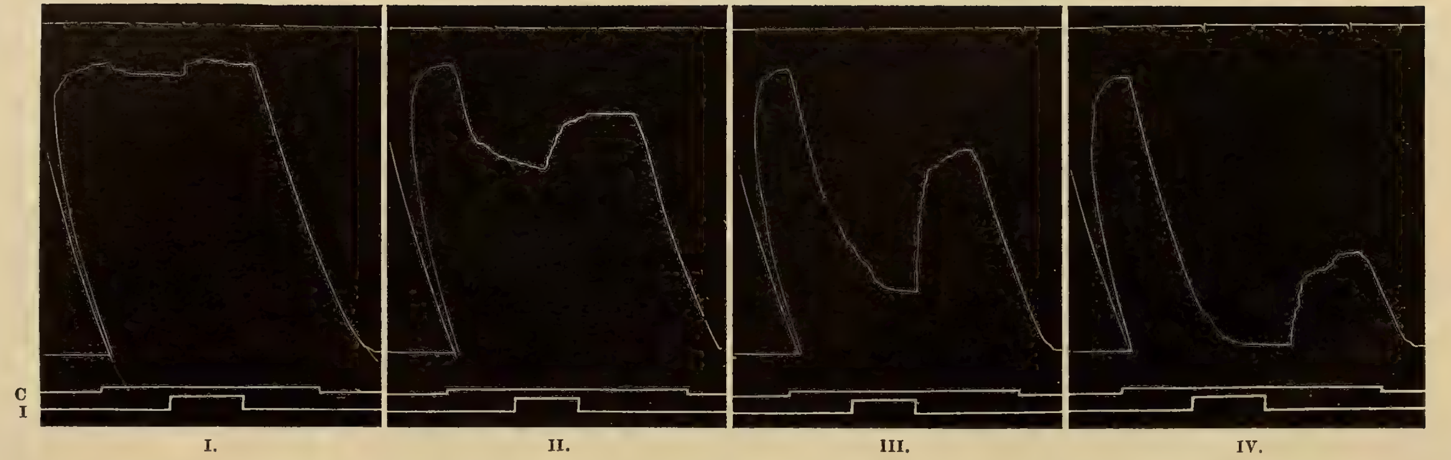

Fig. 5.—Reflex contractions of Semitendinosus evoked by stimulation of the ipselateral peroneal (I) nerve, the intensity of this stimulus being the same throughout the four successive observations. The contraction is inhibited in each observation by an intercurrent stimulation of the contralateral popliteal nerve (C), and the intensity of this latter's stimulus is increased from observation to observation through the series. Cat, decerebrate preparation. Time in seconds above.

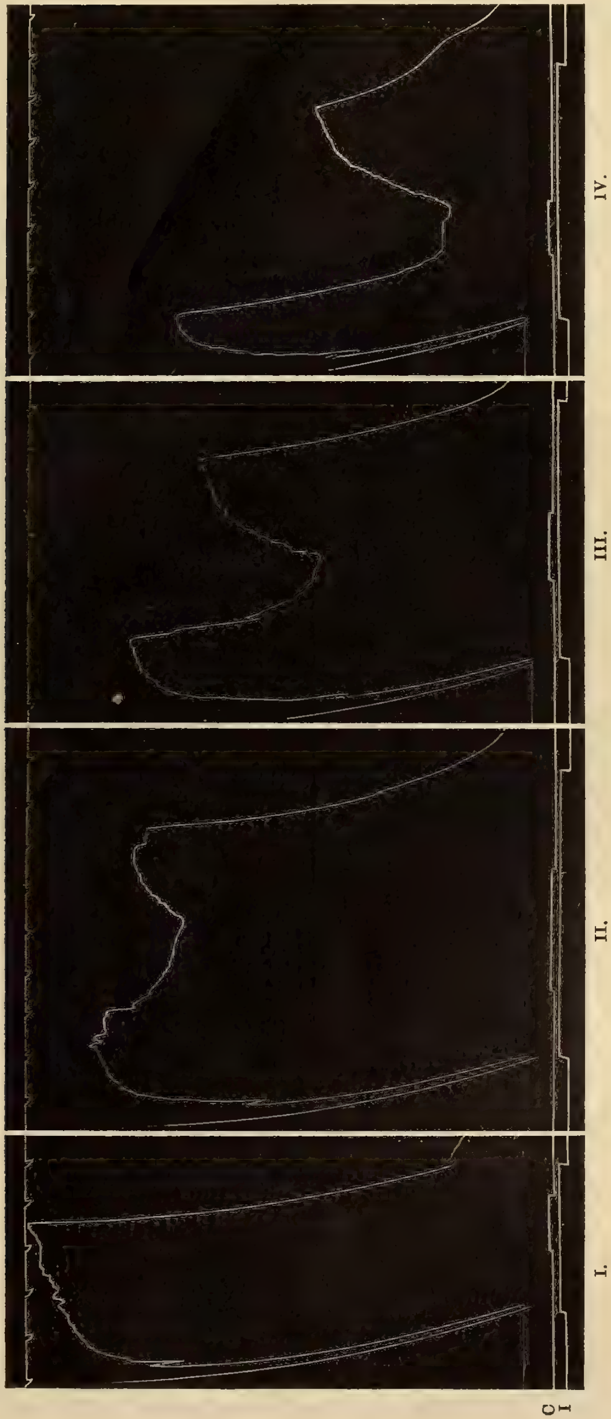

Fig. 6.—Same as fig. 5, except that the intensity of the ipselateral stimulus (I) is varied from observation to observation, being lessened from Observation I to Observation IV progressively, while the intensity of the inhibitory stimulus (C) is kept constant throughout.

excitation with any particular value of inhibition results in a grade of contraction less than that which the excitatory stimulus without the inhibitory would give, and that this grade of contraction is fairly quickly reached, and that then this grade continues unaltered provided there is no alteration in the antagonistic stimuli. In other words, the myograph traces under the combined stimulation a plateau (figs. 5 and 6) following on a brief descent. The same plateau is seen when combined excitatory and inhibitory stimulation follows on purely inhibitory stimulation (fig. 7). This plateau indicates that under the combined stimuli a condition of equilibrium is reached, the two opposed reflex influences of excitation and inhibition balancing at a certain level of contraction or relaxation, the length of the muscle then remaining constant. This result is such as might be expected. It is a result, however, which in my experience is not obtained when reflex inhibition, however feeble, is pitted against that kind of reflex excitation which the muscle (extensor) expresses as tonus. A tonus preparation of the extensor muscle can be obtained by decerebration producing decerebrate rigidity. The relaxation caused in such a preparation by reflex inhibition progresses so long as the inhibitory stimulus continues (fig. 8). The weaker the inhibitory stimulus the slower the rate of relaxation, but the relaxing of the muscle progresses, so long as the inhibitory stimulus continues, until the full degree of relaxation and the *post-mortem* length of the muscle is gradually reached

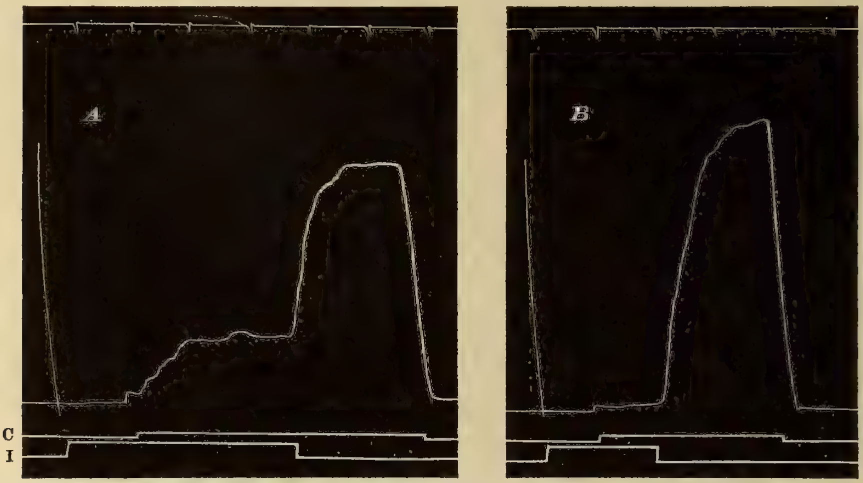

Fig. 7.—Reflex contraction of Vastocrureus. The contraction is of two grades in each observation, according as there is concurrent stimulation of inhibitory and excitatory afferent, and of excitatory afferent alone. C denotes time of stimulation of contralateral peroneal nerve, I of ipselateral peroneal. Cat, decapitated preparation. Time in seconds.

(fig. 8). In other words, a balance between the reflex inhibition and the reflex excitation which tonus expresses is never attained.

This state of tonus appears due to a natural stimulation of mild steady quality, and in decerebrate rigidity it is, as has been shown, *autogenous* in the sense that its afferent path is constituted by proprioceptive nerve-fibres arising in the muscle itself.* This autogenous tonus no artificial stimulus such as we apply to afferent channels experimentally for evoking reflexes has as yet succeeded in giving. The failure to exactly balance the natural reflex excitation which maintains this tonus by any artificial

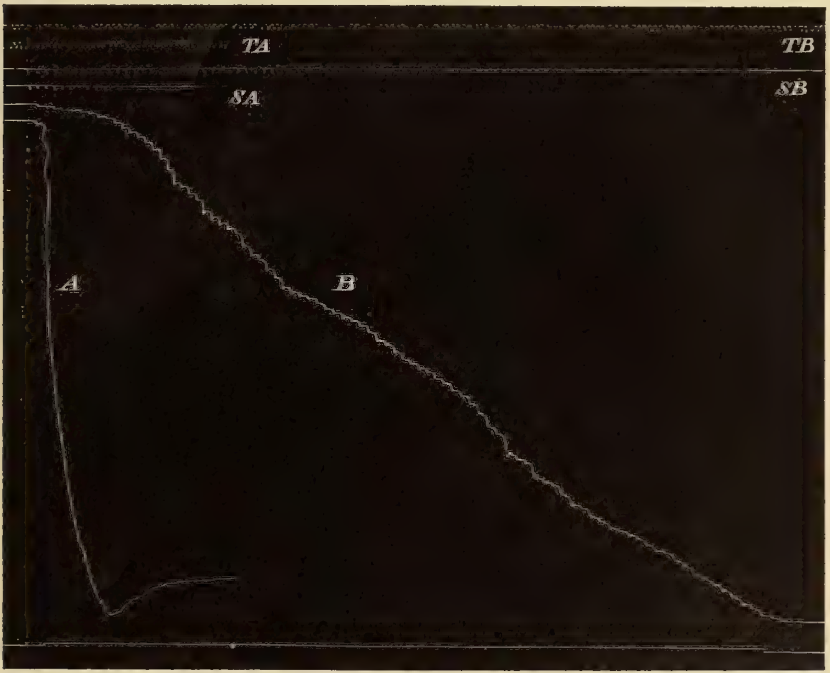

Fig. 8.—Vastocrureus preparation in Decerebrate Rigidity. Reflex inhibition is produced by stimulation of the central end of the severed ipselateral peroneal nerve below knee, the stimulus being a series of slowly repeated break induction shocks, shown by the signal lines SA and SB. Time is marked (TA, TB) in fifths of seconds. In observation A the break-induced currents were more intense (100 units of Kronecker scale) than in observation B (20 units of Kronecker scale). Cat, decerebrate preparation. In both the observations the relaxation of the muscle proceeds to the full resting length of the muscle, but in observation A the fully relaxed condition is obtained speedily in the course of nine repetitions of the stimulus; whereas in observation B the same degree of relaxation is reached only gradually in the course of eighty-six repetitions of the (weaker) stimulus. The signal line at the foot of the figure marks the duration of the stimulation in observation B.

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* Sherrington, 'Quart. Journ. of Expt. Physiol.,' vol. 2, p. 109.

stimulation applied to inhibitory afferents may simply mean that we fail by artificial inhibitory stimuli to induce a reflex inhibition of such a kind as a natural inhibitory tonus would be, just as we fail by artificial excitatory stimuli to induce a reflex contraction of such a grade as is the natural excitatory tonus. In light of this consideration the probability appears that just as there exists a natural excitatory tonus, *e.g.*, the state of the extensors and of some adductors in decerebrate rigidity, so likewise there is a natural reflex inhibitory tonus, but that artificial stimuli such as we have at command at present fail to reproduce it. It may be that the relative slackness of the flexor muscles, in other words their exclusion from excitatory tonus, in decerebrate rigidity is the expression of an inhibitory tonus. Decerebrate rigidity is, as I have shown elsewhere,* to be regarded as a postural reflex, namely, the reflex of standing. If the flexors are under an inhibitory tonus in that reflex condition while the extensors are under an excitatory tonus, it becomes clear that reciprocal innervation applies in this static postural reflex as well as in the ordinary reflexes which execute movements and changes of posture.

In the myograph records (fig. 8) which illustrate failure to obtain balance between the natural reflex tonus of the extensor and the artificially excited reflex excitation there occurs a further feature. The rate of elongation of the tonic muscle under the weak artificially excited inhibition continues about the same from start to finish, even when the starting point is with the muscle fairly fully shortened and the end point is with the muscle fully relaxed. In other words, the degree to which the reflex inhibition overbalances the natural excitation causing the tonic contraction seems practically the same throughout. But the intensity of the stimulation of the inhibitory afferent in these observations was kept the same from start to finish; hence the inference is that the intensity of the natural reflex excitation causing the tonic contraction is about the same throughout. That is to say, the intensity of the excitatory tonus is practically the same when it keeps the extensor muscle so shortened as to maintain the knee fully extended as when the muscle under the tonus maintains the knee at an angle of *e.g.* 60°. In other words, the intensity of the reflex tonus of the extensor may be practically the same while the muscle maintains postural lengths widely different. Thus the same conclusion is reached as has been arrived at by other observations which I have published before.†

IV.—The extensor muscle of the knee (*vastocrureus*) and the flexor muscle

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* 'Integrative Action of the Nervous System,' London and New York, 1906.

† Sherrington, 'Roy. Soc. Proc.,' Note XII, August, 1908; and 'Quart. Journ. of Expt. Physiol.,' *loc. cit.*

(*e.g. semitendinosus*) of that joint being suitably isolated, the myograph can be arranged to register simultaneously the state of contraction or relaxation of both muscles. If, then, excitatory and inhibitory afferents for each of these muscles be stimulated concurrently, summation of the opposed influences of the antagonistic nerves is found to be exhibited concurrently by both muscles. The results of double (excitatory together with inhibitory) stimulation on the antagonist muscles can be grouped into cases belonging to three types. Which particular one of these three types occurs in any particular case depends on the relative intensity of the stimulation of the two afferent nerves. In my experiments usually one of the two nerves has been ipselateral, that is, belonging to the limb to which the observed muscles belong; the other contralateral, that is, belonging to the opposite fellow limb. The ipselateral nerve excites the knee flexor and inhibits the knee extensor (fig. 9A, Obs. I and II). The contralateral nerve excites the knee extensor and inhibits the knee flexor (fig. 9A).

Under concurrent stimulation of both ipselateral and contralateral nerve one type of result is that the knee flexor contracts and the knee extensor is relaxed apparently fully (fig. 9B, Obs. V; fig. 11, Obs. I and II). This is the result when the stimulation of the ipselateral afferent is strong and that of contralateral relatively weak, though yet efficient to cause contraction of extensor muscle were not the ipselateral stimulus concurrently at work.

In a second type of case the result of the concurrent stimulation of the opposed afferents is that knee extensor contracts and knee flexor is relaxed apparently to the full (fig. 5, Obs. IV). For this result the stimulation of the contralateral afferent has to be strong and that of the ipselateral quite weak, though strong enough to relax the extensor and contract the flexor were not the contralateral stimulus in action at the time.

In combinations belonging to the above two types, one of the antagonist muscles contracts while its opponent, if previously at rest, does not enter into contraction, or, if previously contracting, is thrown out of contraction and relaxed, exhibiting no obvious contraction either to visual inspection or in the myograph record. The result in these two types of instance, although obtained under concurrent stimulation of both afferents, resembles to outward appearance that of stimulation of either the one or the other of the antagonist afferents alone.

If, however, the relative intensity of stimulation of the two afferent nerves differs less than in the two sets of cases mentioned, the result of the concurrent stimulation of the two nerves falls out differently. In this third type of case the antagonistic muscles, flexor and extensor, both exhibit obvious contraction concurrently (fig. 9, Obs. II and IV; and fig. 10). Thus the Observation II, FIG. 9, A.

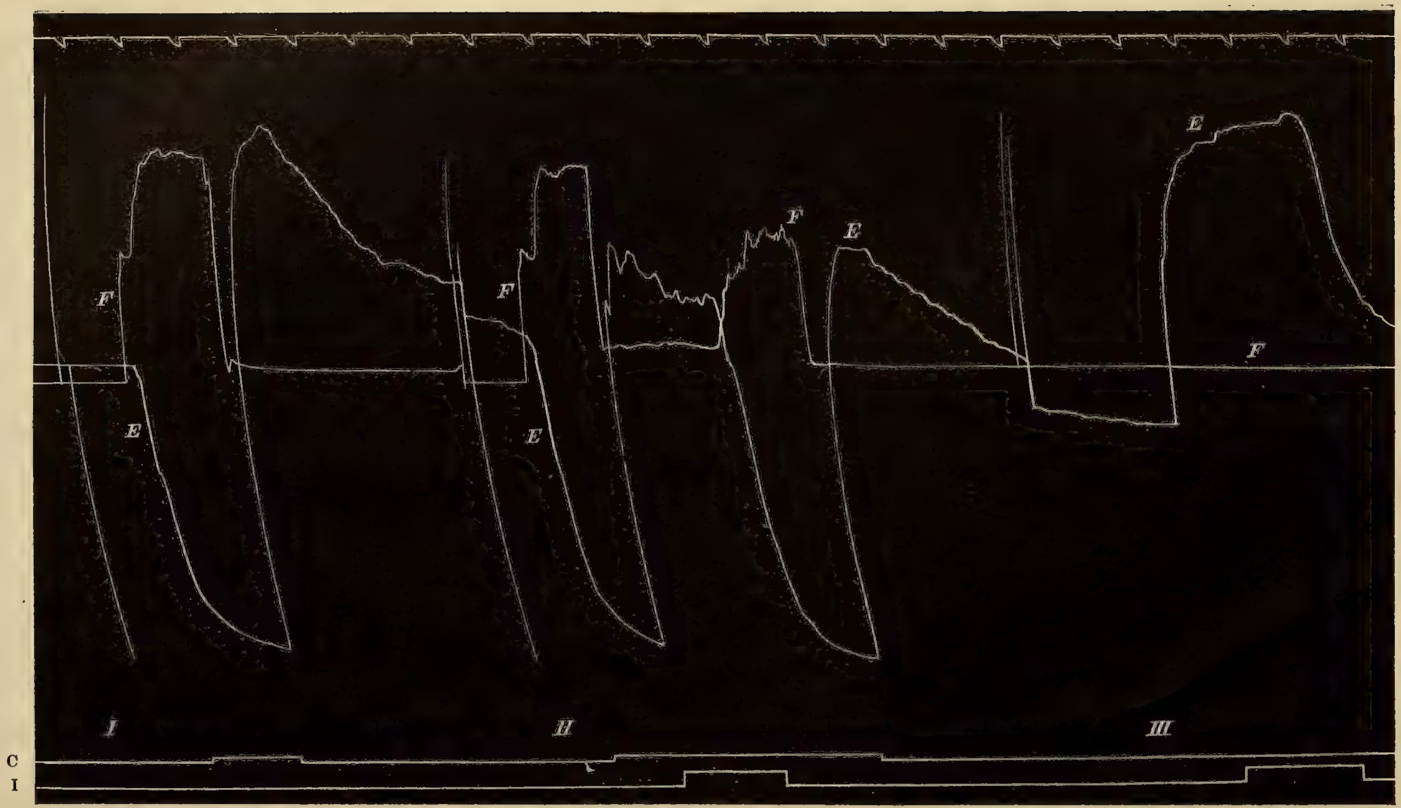

FIG. 9, A and B. Five consecutive Double-myograph Observations. E marks the myograph line of vastocrureus (extensor), F that of semitendinosus (flexor). I marks the signal of the stimulation of the ipselateral afferent nerve (peroneal), C that of contralateral. The observations I, II, III, IV, and V were taken consecutively at intervals of 1 minute. Cat, decerebrate preparation. Time in seconds above. Description in text. The lever attached to vastocrureus (E) writes not directly below but a few millimetres to right of that attached to semimembranosus (F); the distance between them is shown by the control arcs cut by the levers when the recording surface was not moving.

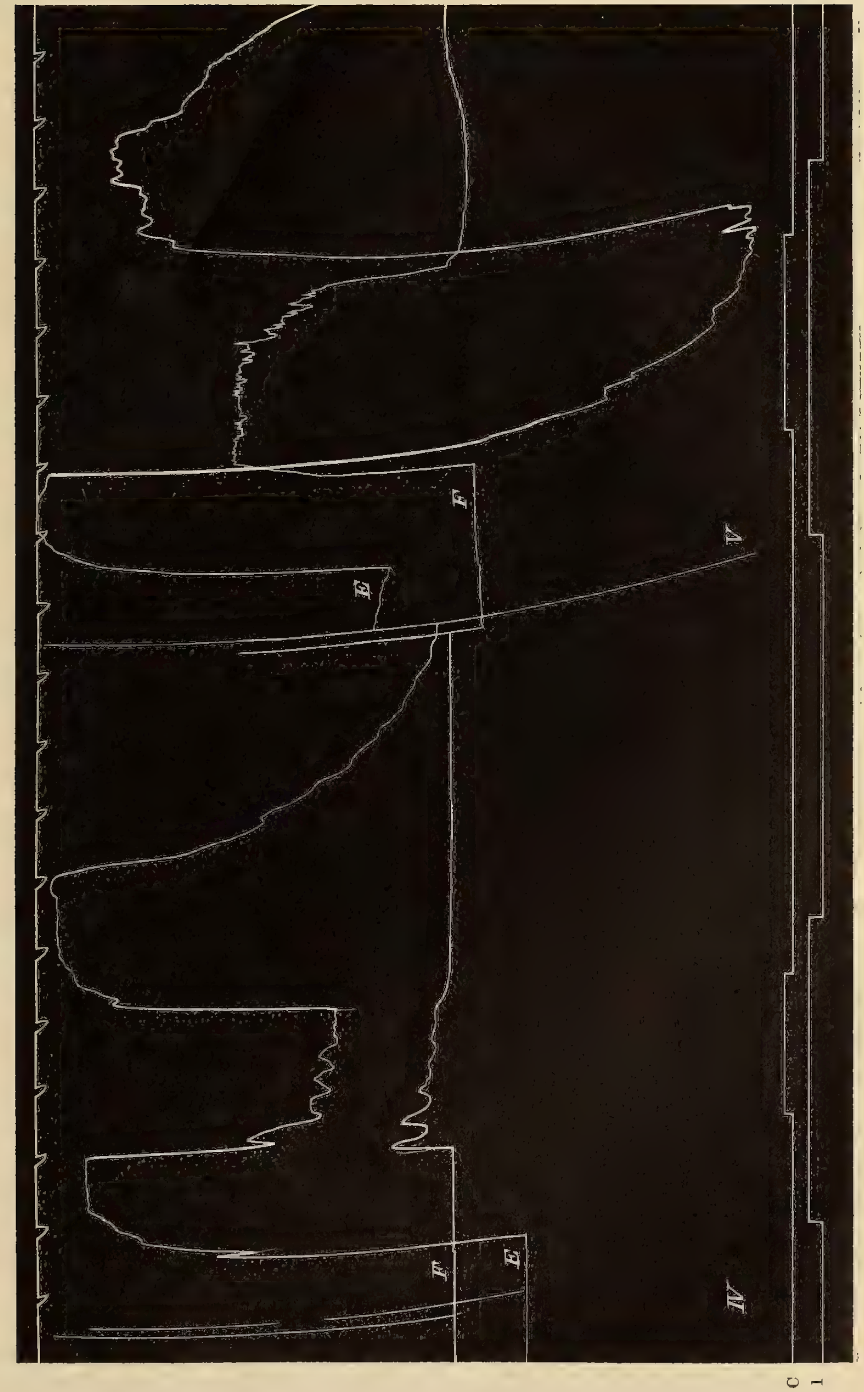

Fig. 9, B.

fig. 9A, opens with stimulation of the ipselateral afferent. This causes contraction of the flexor and, at the same time, relaxation of the extensor. Some-

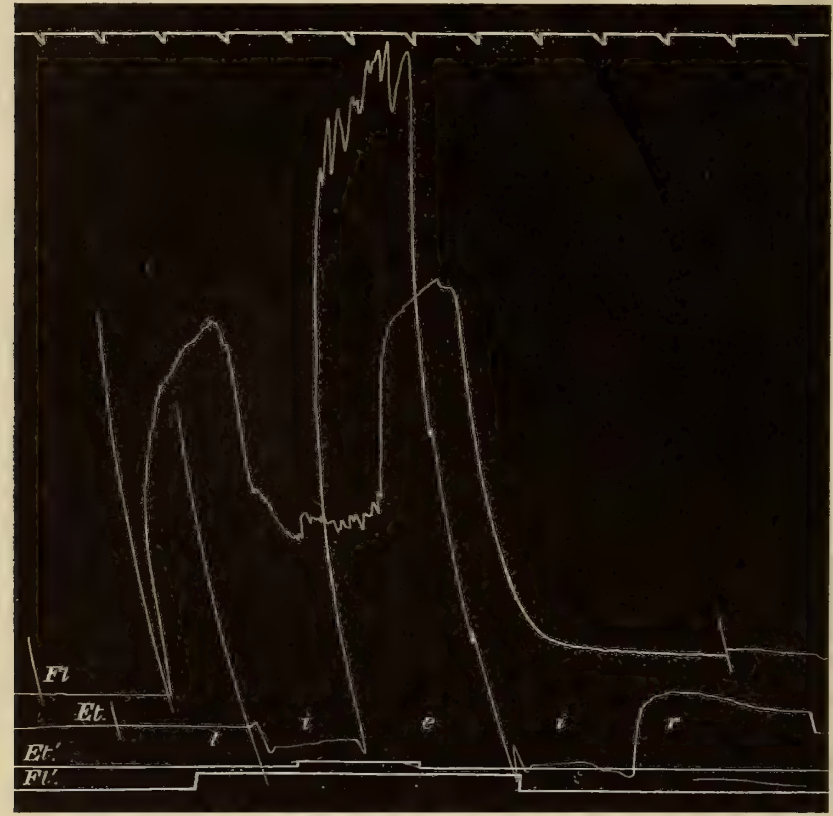

Fig. 10.—Reflex contraction of Semitendinosus, Fl, and Vastocrureus, Et, recorded with double-myograph. The lever attached to vastocrureus (Et) writes not directly below but about a centimetre to the right of that attached to semitendinosus (Fl); control arcs, cut when the recording surface was not moving, indicate the moment of commencement of stimulus on each myogram line. The ipselateral peroneal nerve (afferent end) is stimulated as shown by the lower signal line Fl′, and causes contraction of semitendinosus and relaxation of vastocrureus. Then follows stimulation of contralateral peroneal nerve as shown by the upper signal Et′. This combined stimulation lessens, but not to extinction, the contraction of semitendinosus and at the same time brings into contraction the antagonistic muscle, vastocrureus. On reverting to the single stimulation again, the contraction of semitendinosus increases once more and that of vastocrureus disappears. Finally, on cessation of the single stimulation Fl′, a rebound, *r*, from inhibitory relaxation to increased tonic contraction occurs in vastocrureus synchronously with the relaxation of the flexor. Time above in seconds. Decerebrate cat.

what more than a second later stimulation of the contralateral afferent was commenced, the stimulation of the ipselateral afferent continuing unaltered.

Under this concurrent stimulation of both nerves the extensor muscle at once contracts and the contraction of the flexor muscle continues, but in much diminished degree. About two seconds later the stimulation of the contralateral afferent is discontinued, that of the ipselateral remaining as before. The contraction of the extensor at once dies out, and the flexor resumes the higher degree of contraction, approximately, which it exhibited before the stimulation of the contralateral nerve set in. Finally the stimulation of the ipselateral afferent itself is withdrawn; the contraction of the flexor ceases forthwith, and the relaxed extensor then exhibits a rebound contraction, regaining its tonus, and exhibiting, indeed, a greater tonic shortening than it possessed before it was subjected at all to inhibition.* It is obvious from the record that the flexor muscle and the extensor, during the concurrent stimulation, are in contraction both of them at the same time, and that the height of contraction of the flexor is less under the twofold stimulation than under that of the ipselateral afferent alone. That is evident not only from comparison with the flexor contraction in the first and third parts of Obs. II itself, but from comparison with Obs. I, where the behaviour of the two muscles under the stimulation of ipselateral afferent alone with the same intensity of stimulus as in Obs. II is seen. Observations I, II, and III are successive observations taken at one minute interval. In Obs. I the ipselateral afferent alone was stimulated, and in Obs. III the contralateral afferent alone. Returning to Obs. II, although there is clear evidence from it that the contraction of the flexor during the concurrent stimulation of the antagonist nerves is an algebraic resultant of excitation and inhibition values, what is not so evident is that the concurrent contraction of the extensor similarly exhibits an inhibitory component. That this is, however, really the case is quite clear on comparing Obs. II with Obs. III. In Obs. III the contralateral afferent alone was stimulated. This stimulation has no obvious effect on the flexor muscle (see above, Section I); on the extensor it has the effect of exciting a contraction. The intensity of stimulus applied to the nerve was unaltered from that of Obs. II. But the height of contraction evoked is much greater in Obs. III than in Obs. II. The height of contraction has, of course—this being a tonus preparation of the extensor—to be reckoned, not from the particular grade of contracted state which the tonic muscle happens to be exhibiting at the moment of excitation, but from the base line of full relaxation, such as is revealed under the inhibitions in Obs. I and II. The greater intensity of the extensor's contraction in III than in Obs. II proves that in Obs. II the extensor's contraction is less than it is under the same intensity of stimulus applied to the contralateral afferent alone and that it is less by an amount represented by the difference between the height of contraction in Obs. II and Obs. III respectively. Comparison of Obs. II with Obs. III furnishes, therefore, one proof that under the concurrent stimulation the contraction of the extensor like that of the flexor shows algebraic summation of neural states of unlike sign. And comparison of Obs. II with Obs. IV (fig. 9B) furnishes further proof of the same thing. Obs. IV is a repetition of Obs. II, the intensities of the stimuli being the same in Obs. II, but the sequence of the stimuli in Obs. IV is in reverse order to their sequence in Obs. II. Obs. IV opens with stimulation of the contralateral afferent; this stimulation produces contraction of the extensor with no obvious effect on the flexor, the preparation not being a tonus preparation as regards the flexors. After the contralateral stimulus has been in operation for rather more than a second the ipselateral afferent is stimulated, the stimulation of the contralateral continuing as before. The effect of the concurrent stimulation of the two nerves is that the contraction of the extensor is considerably reduced in height, and that a contraction of the flexor occurs, but this contraction of the flexor is much less than that given by the ipselateral stimulus acting alone as in Obs. I and Obs. II. After about 2 seconds' duration the ipselateral stimulus is withdrawn, there then ensues immediately a disappearance of the flexor's contraction and a resumption by the extensor of the full height of contraction it showed originally under contralateral stimulus alone. Finally the contralateral stimulus is withdrawn and the contraction of extensor soon begins to decline and somewhat gradually passes off. The difference between the abrupt reduction of the extensor's contraction directly the ipselateral stimulus (inhibitory) joined the contralateral and the delayed and gradual dying out of the contraction on mere withdrawal of the exciting stimulus (contralateral) is characteristic of the difference between onset of inhibition and mere cessation of excitation in a tonus preparation.

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* Sherrington, 'Roy. Soc. Proc.,' Note IX, 1906.

Fig. 9B, Obs. V, taken a minute later than Obs. IV, supplies a repetition of the latter with increased strength of the ipselateral stimulus. The result then given during the concurrent stimulation of the opposed afferents is different. The simple change in the intensity of stimulation of one of the afferents removes the result from the type 3 to type 1. Under the concurrent stimulation in this observation the flexor contracts while simultaneously the contraction of the extensor is apparently completely suppressed, whereas under the concurrent stimulation in Obs. II and IV both the flexor and extensor are synchronously in active contraction.

In this third type (fig. 9, Obs. II, Obs. IV, and fig. 10) of result, therefore, concurrent stimulation of the two antagonistic afferents produces in the antagonistic muscles obvious contraction concurrently in both. But though both the muscles are contracting the contraction of each is seen to be less than it would be were there not then in action the reflex which produces contraction of its antagonist. And this is so because some reflex inhibition of the motoneurones of each accompanies the reflex excitation of the motoneurones of the other. Thus the contraction of semitendinosus is less than it would be were not the contralateral afferent being concurrently stimulated, and the contraction of vastocrureus is less than if the ipselateral afferent were not concurrently stimulated. As to which of the two antagonists exhibits the greater contraction, that feature is determined by the relative intensity of the stimulation of the two antagonistic afferents. To obtain the type 3 of result the effects of the two opposed afferents have, of course, to be sufficiently nearly balanced, and the intensity of the one must not wholly overbalance that of the other. Within these limits, however, all degrees of grading of combination of the two seem obtainable by simple adjustment of the relative intensity of the stimulation of the opposed nerves. In short, a delicate grading of the intensity of contraction is effected by bringing into play a double reciprocal innervation. The result of the double reciprocal action is that each of the two muscles displays simultaneously with its antagonist an algebraic summation of reflex inhibition and reflex excitation. In other words, antagonistic reflexes add themselves algebraically with unlike signs.

To obtain this result with spinal reflexes, *e.g.* in the decapitated mammal, or with reflexes produced by stimulation of afferent nerves in the decerebrate animal, it is necessary to stimulate synchronously two or more afferents of opposed effect as regards the antagonist muscles selected. Stimulation of either of such opposed afferents taken alone never suffices, in my experience, to produce simultaneous contraction, *e.g.* of the knee flexor and extensor. In short, concurrent contraction of the antagonists denotes that two antagonistic reflexes are synchronously at work and are operating with intensities not far removed from balancing one another. In this respect there is a significant resemblance between spinal reflex co-ordination and that exercised by the motor *cortex cerebri*. Electric stimulation at one cortical point giving contraction of, *e.g.* flexor of elbow with relaxation of the antagonist extensor, and stimulation at another cortical point giving contraction of the antagonist extensor with relaxation of the flexor, synchronous stimulation of the two cortical points together does often, I find, give, if suitably graded, a concurrent contraction of both flexor and antagonist extensor together.

V.—Apart from the intensity of the stimuli applied to the opposed afferent nerves there is, however, another factor controlling the balance of reflex result on the antagonistic muscles. It was shown previously that the ipselateral reflex on the knee muscles is, as compared with the crossed reflex, the prepotent one. Remembering that the influence of the ipselateral afferent is excitation of the flexor and inhibition of the extensor, while that of the contralateral afferent is inhibition of the flexor and excitation of the extensor, it has been shown that each of these afferent nerves can be made to preponderate in reflex result over the other. This preponderance in effect of the one over the other can be impressed on either the one or the other of the two by simply stressing the intensity of the stimulus to the one it is desired should preponderate. But it is less easy to secure prepotency for the contralateral nerve than for the ipselateral. The ipselateral reflex seems to function with an inherent greater intensity than the contralateral. Fig. 11 exemplifies the effect that this has on the experimental adjustment of the stimuli required for algebraic summation of excitation and inhibition. The observation opens with stimulation of the contralateral afferent which produces contraction of the extensor muscle and no obvious effect on the flexor, the preparation not being a tonus preparation as regards the flexor. Two seconds later there ensues stimulation of the ipselateral afferent, stimulation of the contralateral continuing unaltered; the flexor at once contracts, and the contracting extensor is at once relaxed, and so speedily and fully that its lever gives a vibratory shake after the fall. A second and a half later the ipselateral stimulus is withdrawn; the contraction of the flexor then dies off, and the contraction of extensor reappears and regains its

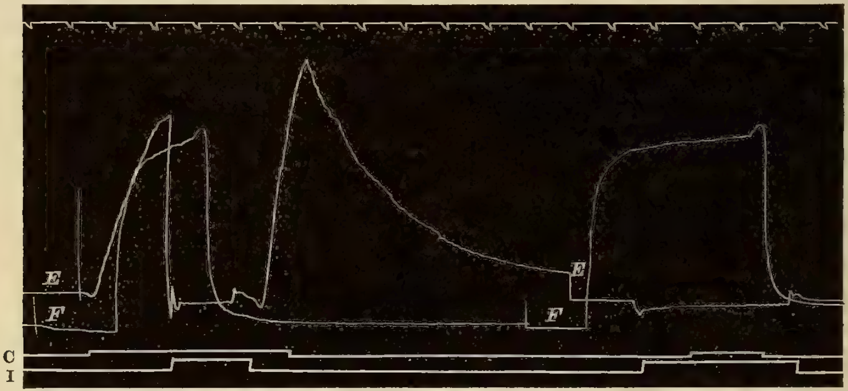

Fig. 11.—Double-myograph Observation. E marks vastocrureus, F semitendinosus, C contralateral stimulus, I ipselateral stimulus. Cat, decerebrate preparation. Time in seconds. Description in text. The myograph lever attached to vastocrureus writes not directly above that attached to semitendinosus, but about a half centimetre to right of it; arcs cut by the levers on the recording surface when stationary mark the moment of application of stimulus on each myograph line.

old prominence. Finally, the contralateral stimulus is withdrawn and the extensor's contraction gradually passes away. After a minute's pause the observation was repeated with unaltered intensity of the stimuli, but with reversal of their sequence. The ipselateral stimulus precedes and produces its unopposed effect; this stimulus still remaining in operation, the contralateral stimulus is now combined with it and remains without visible influence on the myogram, although what its unopposed effect would be is guaranteed by the just preceding observation. This comparatively easier suppression of the contralateral reflex by the ipselateral than of the ipselateral by the contralateral explains how it is that when similar afferent nerves of both hind limbs or similar points of skin in the limbs are simultaneously and similarly stimulated extension occurs in neither limb and flexion occurs in both.*

The effect of fairly strong and equal concurrent stimulation of symmetrical afferents of the two limbs in giving flexor effect at both knees, whereas stimulation of one of the afferents gives flexion at ipselateral knee and extension at contralateral, recalls strikingly a result obtained by Mott and Schäfer† on the eyeball movements under concurrent stimulation of balanced points of the two cerebral hemispheres right with left. Stimulation of the cortex of one hemisphere gives lateral deviation of both globi to the opposite side. It has been shown‡ that in this movement reciprocal innervation is at work producing inhibition of one muscle with excitation of its antagonist. Mott and Schäfer found concurrent stimulation of points in the two hemispheres produce, when appropriately graded, a convergence of the two globi. Here, obviously, of the two antagonistic influences, that on the ipselateral globus preponderates over that on the contralateral, just as in the case of the limbs the ipselateral reflex preponderates over its antagonistic contralateral. And in the eyeball result, just as in the result on the limbs, I imagine that appropriate analysis would reveal in this case an algebraic summation of excitation and inhibition just as with the flexor and extensor knee muscles. In short, in both cases antagonistic reactions are adding themselves with *plus* and *minus* signs.

VI.—It will be noted that the ipselateral afferent, just as it is prepotent in excitation, so also is prepotent in inhibition. This supports the view put forward in these Notes§ that the inhibition and the excitation are complemental parts of one and the same reflex. In harmony with this stands also the fact that when the one or the other afferent is made prepotent by suitably increasing the intensity of its stimulus, the prepotency obtains both for excitatory influence and inhibitory influence alike. The reflex produced by either afferent is, in fact, a reflex of simultaneous double sign (± reflex). And in the observations furnished there is a good deal of evidence suggesting that the relation between the intensity of the excitatory part of the reflex and the inhibitory is such that the intensity of the one increases proportionally with that of the other. The rhythm of the inhibitory effect in the motoneurones of one muscle appears also to be synchronous with that of the excitatory effect in the motoneurones of the antagonist muscle, to judge by the rhythmic tremor not unfrequently evident on the myograph tracings. Observation IV of fig. 9B furnishes an illustration of this.

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* Sherrington, article "Spinal Cord," 'Schäfer's Text-book of Physiology,' vol. 2, p. 840, 1899; also 'Integrative Action of Nervous System,' p. 225, 1906.

† 'Brain,' vol. 13, p. 170, 1894.

‡ Sherrington, 'Roy. Soc. Proc.,' Note II, vol. 53, p. 411, 1893.

§ Sherrington, 'Roy. Soc. Proc.,' Note VIII, vol. B 76, p. 277, 1905.

VII.—It was shown previously that after reflex inhibition of the motoneurones of the extensor muscle there ensues, in decerebrate rigidity, on withdrawal of the inhibitory stimulus, a contraction of the muscle. This is the rebound contraction which I attribute to "successive spinal induction." It may far exceed in intensity the tonic contraction which existed prior to the inhibition. The question arises as to what state obtains in the flexor muscle when this rebound contraction of its antagonist occurs. With the double myograph recording both extensor and flexor simultaneously, this relation can be studied. Fig. 9A, Obs. I and II, and fig. 10, *r*, give instances of the time relations observable. On withdrawal of the ipselateral stimulus, the flexor contraction passes off, and at the same time there takes place the rebound contraction of the extensor. Often a relatively long latency period intervenes between the cessation of the stimulus and the onset of the extensor's rebound contraction. Similarly it is not unusual for the contraction of the flexor to outlive the duration of the excitatory stimulus for a certain time, the flexor motoneurones exhibiting after discharge. The simultaneous record of the two muscles shows that the rebound contraction of extensor synchronises with the dying out of the after-contraction of the flexor; the after discharge (rebound) of the extensor motoneurones comes into action as that of the flexor motoneurones passes off. Therefore, in the after-action shown by these antagonistic centres, there is evident the same kind of reciprocal activity as is seen in their reaction under stimuli in application at the time. In after-discharge and in tonus, as also when reacting to ordinary reflex stimulation, reciprocal innervation seems to hold good in regard to the mutual relation between the motor centres of the antagonistic muscles.

The Properties of Colloidal Systems. I.—The Osmotic Pressure of Congo-red and of some other Dyes.

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(Received April 19,—Read May 6, 1909.)

Experiments made in 1895 by Linder and Picton* upon solutions of arsenious sulphide indicated that colloidal solutions possess a real osmotic pressure, although the authors themselves claim no quantitative value for their results. In 1905† further experiments were made, but again great difficulties were met with, and, although it seemed evident that osmotic pressure was present, the numerical values obtained were irregular and small.

The first definite proof that certain colloidal solutions are able to exert a not inconsiderable osmotic pressure was given by Starling‡ in the case of the colloids of blood-serum. When separated by a gelatin membrane from a solution obtained by filtration of some of the same serum through Martin's gelatin filter, the pressure rose to about 30 mm. of mercury.

Waymouth Reid§ found that solutions of carefully purified hæmoglobin gave an undoubted osmotic pressure when separated from water by a membrane of parchment-paper, but regards this fact as evidence that hæmoglobin forms a true solution.

Moore and Parker‖ determined the osmotic pressures of the colloids of white of egg, of serum, and of soap solutions, while Moore and Roaf¶ made measurements of those of serum-proteins, of gelatin, and of gum-acacia.

Hüfner and Gansser** and, somewhat later, Roaf,†† independently of one another, made careful determinations of the osmotic pressure of hæmoglobin solutions, to which reference will be made in a subsequent page.

It will be noticed that, in all these cases, with the exception of arsenious sulphide and soaps, the chemical constitution of the body investigated is uncertain, although the molecular weight of hæmoglobin has been calculated from its content in iron. The experience of those who had worked with arsenious sulphide and with soaps was not encouraging for further research, so that it seemed desirable to investigate the behaviour of colloids of known chemical constitution and molecular weight, the latter to be as small as possible, in order that the osmotic pressure should be sufficiently great. By this means it might be possible to estimate the number of molecules taking part in the formation of "solution-aggregates" or colloidal elements, and also to obtain more definite information as to the effect of electrolytes on the osmotic pressure.

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* 'Chem. Soc. Trans.,' vol. 67, p. 72, 1895.

† 'Chem. Soc. Trans.,' vol. 87, p. 1909, 1905.

‡ 'Journ. Physiol.,' vol. 19, p. 322, 1896, and vol. 24, p. 318, 1899.

§ 'Journ. Physiol.,' vol. 33, p. 12, 1905.

‖ 'Amer. Journ. Physiol.,' vol. 7, p. 261, 1902.

¶ 'Biochem. Journ.,' vol. 2, p. 34, 1907.

** 'Archiv f. Physiol.' (Engelmann), 1907, p. 209.

†† 'Physiol. Soc. Proc.,' 1908, p. i, in 'Journ. Physiol.,' vol. 39, 1909.

Certain of the aniline dyes form colloidal solutions, if we may take Graham's criterion of non-diffusibility through parchment-paper as decisive. One of these dyes is congo-red, whose constitution and molecular weight are well known. My attention was first called to the fact that solutions of this body have a considerable osmotic pressure by phenomena met with in purifying it by dialysis. It was striking and, in fact, a matter of some inconvenience, to find that the contents of the parchment-paper tubes rapidly increased in volume by taking up water and, unless some of the fluid was removed, continuously overflowed. Congo-red, therefore, formed the starting-point of the observations to be recorded in the present paper.

Owing to their high colouring power, the aniline dyes present many advantages for the study of colloidal properties. In the investigation of osmotic pressure, for example, the slightest leak in the membrane of the osmometer is detected at once.

The particular form of osmometer used was that of Moore and Roaf,* modified in order to change at will the fluid on the side of the membrane opposite to the solution under investigation. Repeated changes of distilled water could be made until no further change in the osmotic pressure occurred, while the effect of the presence of various electrolytes or other bodies could be examined. A diagram of the apparatus is given in fig. 1.

Congo-red, as obtained from Kahlbaum, was found to contain an appreciable amount of sodium chloride. In order to remove this, hydrochloric acid was added until the red colour had vanished and the free acid, precipitated, was washed on a filter with distilled water. It was soon found that the free acid went into a beautiful deep blue colloidal solution, which passed through the filter. (This observation has been published by Pelet-Jolivet and Wild,† since my experiments were made.) I was obliged, therefore, to resort to prolonged dialysis against distilled water. This dialysed solution was placed in the osmometer. It gave a very small osmotic pressure, about 6 mm. Hg. Subsequent experiments, to be described below, were made to determine the osmotic pressure of this blue colloid more accurately. Dilute solution of sodium hydroxide was then run through the lower chamber in order to convert the free acid into the sodium salt. This solution was replaced at intervals of 24 hours until the outside solution remained permanently slightly alkaline. During this process the osmotic pressure rose gradually to about 40 mm. Hg. Repeated changes of distilled water were then similarly run through as long as the pressure continued to rise. After ten days, equilibrium was attained with a pressure of 79·3 mm. Hg at a temperature of 30°·2 C.

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* *Loc. cit.*

† 'Kolloid-Zeitsch.,' vol. 3, p. 175, 1908.

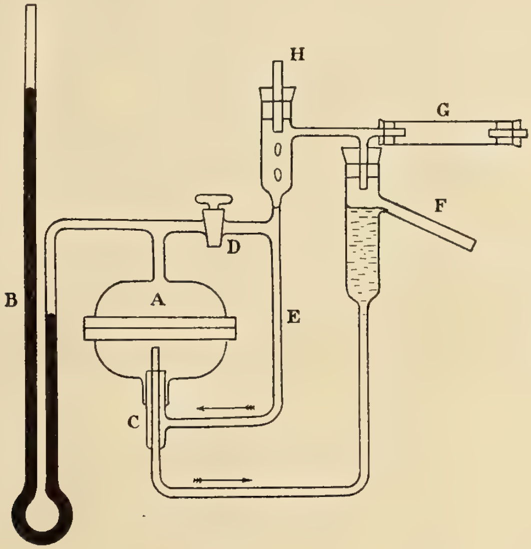

Fig. 1.

A, Osmometer of Moore and Roaf.
B, Mercury manometer, read by means of a reading microscope.
C, T-tube, with inner smaller tube, to allow a current of water, or other fluid, through the outer, lower, chamber of the osmometer.
D, Tube, connecting the two chambers when the stopcock is opened. This is done in order to control the zero of the manometer at any time.
E, Inlet tube to the lower chamber. F, Outflow.
G, Soda-lime tube to exclude atmospheric CO_2.
H, Tube from flask of distilled water or other fluid.

The osmometer was immersed in a thermostat.

The molecular weight of congo-red (the di-sodium salt of benzidine-tetrazo-di-naphthylamine-di-sulphonic acid) is 696·47. On the basis of an osmotic pressure of 22·4 atmospheres for a molar solution (as true solution) at 0°, a 1-per-cent. (= 10 grammes per litre) solution at 30°·2 should have a pressure of

$$22{\cdot}4 \times 760 \times \frac{273 + 30{\cdot}2}{273} \times \frac{10}{696{\cdot}47} = 271{\cdot}4 \text{ mm. Hg.}$$

At the end of the above experiment, the solution was pipetted out of the osmometer and its concentration determined by evaporating to dryness a known volume and drying the residue to constant weight in a toluene bath in the usual way. It was found to be 0·30 per cent., so that, if the dye had been present as separate molecules, the osmotic pressure should have been 0·30/1 × 271·4 = 81·4 mm. Hg. The actual value found was 79·3 mm. Hg, or 97 per cent.

In other experiments the agreement with theory was not so good, *e.g.* 207 mm., instead of a theoretical 228 mm., or 91 per cent., for a 0·84-per-cent. solution at 30°·2; 77·4 instead of 84, or 92 per cent., for a 0·309-per-cent. solution at 30°·7; and 128 instead of 146, or 88 per cent., for 0·58-per-cent. solution at 10°.

It is obvious that these values could only be obtained if the greater part of the elements responsible for the production of osmotic pressure were present as single molecules, since any value greater than one-half the theoretical implies that a part of the active elements consists of single molecules.

When the molecular weight of hæmoglobin is calculated from the content in iron, a value of about 12,000 to 14,000 is obtained.* Now this is the same number obtained from the osmotic pressure determinations of Hüfner and Gansser,† and of Roaf.‡ Hæmoglobin, therefore, exists in solution in single molecules, although, like congo-red, it does not pass through parchment-paper. The molecular weight of congo-red, however, is very much less than that of hæmoglobin, only about one-twentieth in fact, so that it is more surprising to find it to behave as a colloid.

On this account it is advisable to examine how far congo-red exhibits other properties associated with colloids. To what degree does a molecule of such dimensions show the characteristics of matter in mass, possessing surfaces?

In the first place, what appearance does a solution of congo-red show in the ultra-microscope? According to Michaelis§ the particles present are sub-microscopic, that is, resolvable into separate bright points. The same statement is made by Pelet-Jolivet and Wild.|| My observations are not entirely in agreement with those of the investigators mentioned. There are undoubtedly, a few scattered bright points to be seen, but these only

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* See Schultz, 'Die Grösse des Eiweissmoleküls,' p. 31, Jena, 1903.

† *Loc. cit.*

‡ *Loc. cit.*

§ 'Deutsche Med. Wochensch.,' No. 42, 1904.

|| *Loc. cit.*

account for a very small part of the total quantity of the dye present in the solution, as can easily be shown as follows: The blue colloidal free acid of congo-red, even in extreme dilution, shows the track of the beam of light filled with shining points of a beautiful copper colour and of nearly equal size, so far as their diffraction images enable one to judge. If a drop of dilute alkali be added to this solution, the track of the beam suddenly vanishes, occasionally a bright point moves into the field and back again. These few particles seem to be slightly larger than those of the acid. When the illumination is carefully adjusted and made as brilliant as possible, close attention shows that the track of the beam is very faintly visible as a bluish grey haze, not resolvable into separate points, at all events not with the means at my disposal, viz., arc light, Zeiss D* objective. As I am inclined to interpret the phenomena, the faint haze is the optical expression of the part of the dye present in the molecular state, and the rare bright points are due to aggregates of a number of molecules, produced by the action of traces of electrolytes, to which congo-red is enormously sensitive, as will be shown below. The solutions described by previous observers as being resolvable into particles by the ultra-microscope were, in all probability, not sufficiently free from electrolytes.

The ultra-microscope, then, does not throw much light on the nature of solutions of congo-red, since, although it does not contain particles large enough to be visible by means of this instrument, other undoubted colloids, such as ferric hydroxide, are similarly non-resolvable, but show a faint haze.* Moreover, the phenomena described above in the case of congo-red are very like those seen by Michaelis† in certain protein solutions, namely, a part visible as granules and the rest not so resolvable.

The property of carrying an electric charge, not as an ion, but on undissociated molecules, is shared by congo-red with matter in mass. In Whetham's boundary apparatus the dye moves as a whole towards the anode and is, therefore, negatively charged. The origin of the charge is obscure, but is, perhaps, derived from electrolytic dissociation.‡ In accordance with its nature as an electro-negative colloid, congo-red is aggregated or precipitated by cations, especially powerfully by bi- and tri-valent ions. It is also precipitated by an electro-positive colloid, such as toluidine-blue or ferric hydroxide. The precipitate has the properties of an adsorption-compound, in that its composition varies with the relative concentrations of the two colloids present in the solution. As I have shown elsewhere,* the behaviour of congo-red in respect of adsorption by cotton and other materials is that of an electro-negative colloid.

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* Zsigmondy, 'Zur Erkenntniss der Kolloide,' Jena, 1905, p. 148.

† 'Virchow's Archiv,' vol. 179, pp. 205—208, 1905.

‡ The phenomena seen in the boundary apparatus are of some complexity, being accompanied by slight electrolysis. These will more properly form the subject of a separate paper.

The statement is sometimes made that colloids have no definite point of saturation. Congo-red, on the contrary, has, in a certain sense, a somewhat indefinite limit of solubility. It appears, however, that many colloids, especially the inorganic ones, tend to aggregate and deposit when their particles are brought into too close apposition; it may be that traces of electrolytes are responsible for this behaviour, which is thus not the same thing as the crystallisation of a super-saturated true solution.

Recent research tends to show that there is no real line of demarcation to be drawn between colloids and crystalloids. Congo-red is evidently one of those interesting cases which have some of the properties of both classes. In any case, it does not seem reasonable to expect fundamental differences as regards properties dependent on dimensions of the active elements between a large molecule such as congo-red, containing some 70 atoms, and a particle of colloidal gold containing a similar number of atoms. The properties referred to are those dependent on diffusion, such as osmotic pressure and those dependent on surface development.

The fact that, in the case before us, true solution and colloidal solution are one and the same thing suggests several interesting questions. At what molecular size do bodies begin to show properties due to surface development, although still in the condition of single molecules? Again, why should we not be able to reduce the number of molecules in the aggregates of colloidal gold until they consist of single molecules? In this case we should have a true solution of gold in the metallic state, not in the ionised condition. It is possible that differences of electric potential and surface-tension oppose obstacles to such a phenomenon.

The great sensitiveness of congo-red to traces of electrolytes has already been incidentally referred to and this fact makes it a matter of considerable difficulty to obtain the maximum readings of the osmotic pressure as given above. For example, a solution of congo-red, containing 0·84 per cent. of the dye, dialysed against repeated changes of ordinary distilled water until no further rise of pressure took place, gave an osmotic pressure of only 118 mm. Hg, whereas on using water which had been distilled after the addition of potassium permanganate and sulphuric acid and a *second time* after the addition of barium hydroxide, being kept from contact with air by soda-lime tubes, the pressure rose to 207 mm. Hg. The ordinary distilled water used above was a fairly good sample, having a conductivity of not more than 5 gemmhos at 18° while the purer water had a conductivity of 1·8 gemmhos. If still better water had been used, no doubt the full theoretical value of 228 mm. for the osmotic pressure would have been reached.

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* 'Biochem. Journ.,' vol. 1, pp. 175—232, 1906.

Since the ordinary distilled water presumably contained carbonic acid, I tried the effect of water through which carbon dioxide gas had been passed until its conductivity was 23 gemmhos. By titration, this solution was found to contain 0·19 gramme CO_2 per litre. The osmotic pressure fell from 207 to 120 mm. Hg.

The powerful action of so weak an acid as carbonic is rather surprising, and makes it unnecessary to subject stronger acids to detailed investigation.

When the solution of carbonic acid in the above experiment was replaced by a decinormal solution of sodium chloride, the osmotic pressure fell in the course of 24 hours to 15 mm. Hg. When equilibrium was established, the concentration of sodium chloride on both sides of the membrane would be about one-twentieth normal.

Linder and Picton* found that when aggregation of arsenious sulphide was brought about by an electrolyte, it was impossible to reverse the process by washing with distilled water. Similarly, although repeated changes of distilled water were passed through the osmometer after the sodium chloride, until the issuing water gave no reaction with silver nitrate, the osmotic pressure only rose to about three-quarters of its initial value. It is possible that very much more prolonged dialysis might have produced further effect, but it seemed more important to use the apparatus for other experiments, since all these experiments are of necessity of long duration. This washing with water was, in one case, continued for three weeks, and, although after this process the osmotic pressure had risen only to three-quarters of what it was before the action of sodium chloride, no further rise was to be detected on the mercurial manometer when a fresh change of water was added. If a more delicate manometer had been used, it is quite possible that the pressure would have been found to be still rising very slowly, since the extreme slowness of removal of the last traces of electrolyte is characteristic of adsorption phenomena.†

In order to test the action of alkali, a solution of the blue free acid was placed in the osmometer. Only a small pressure of a few millimetres was obtained, too small to read accurately on the mercurial manometer. When dilute sodium or ammonium hydroxide was run into the lower chamber, the pressure rose rapidly as long as the alkali combined with the dye-acid.

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* 'Chem. Soc. Trans.,' vol. 87, p. 1911, 1905.

† Bayliss, 'Biochem. Journ.,' vol. 1, p. 182, 1906.

When excess was present, as shown by the permanent alkaline reaction of the solution, the pressure fell again. The explanation is, no doubt, that the aggregating action of the cation made itself felt. In view of the results of Moore and Roaf* on the augmentation of the osmotic pressure of protein solutions under the action of alkali, the fact noted by me is of some interest. It seems probable that, in the case of proteins, the rise of osmotic pressure is the result of the formation of new colloids by chemical action, these "salts" having smaller "solution-aggregates" than the original colloid.†

If we were unaware of the chemical nature of congo-red and observations were being made of the osmotic pressure of the solution of the free acid, we might imagine that alkali caused a large increase in the osmotic pressure of this body. The fact is that the blue colloidal solution of the free acid, as will be shown later, consists of large particles, easily resolvable by the ultra-microscope, and producing only a small osmotic pressure. When alkali is added, the salt, ordinary congo-red dye, is produced, and this, as shown above, exists in solution in single molecules with high osmotic pressure.

The fact that, as an electrolyte is added in stages, pausing sufficiently long between each addition to allow equilibrium to be established, there is a definite osmotic pressure for each step, so that a continuous smooth curve is obtained, shows that the action of a low concentration of electrolyte must be exerted on a part only of the molecules present. For example, it is not every molecule that unites with another one, since, if so, there would be no intermediate stages between full and half osmotic pressure. From the fact that these stages do exist it follows that a number of molecules are left single. The process is analogous to the association which takes place in ethyl alcohol when dissolved in benzene, where the apparent molecular weight of the alcohol rises from 50 to 208 in regular gradation as the concentration rises from 0·494 to 14·63 grammes to 100 of benzene.‡ This can only be explained by the assumption of a steadily increasing number of molecules becoming associated with others, while the rest remain free.

Ultra-microscopic observations of the actions of electrolytes on congo-red, although somewhat difficult to interpret, confirm the results given by measurements of osmotic pressure A dilute solution, showing only a very few scattered bright points, on the addition of a solution of carbonic acid or a natural salt contains a greatly increased number of these bright particles, which vary considerably in size. In this respect they contrast with the particles seen in the colloidal solution of the free acid, which are strikingly uniform in size. It appears that the action of an electrolyte is, so to speak, selective, leaving some molecules free, while causing others to aggregate into particles, consisting themselves of very different numbers of molecules.

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* *Loc. cit.*, p. 66.

† See also Lillie, 'Amer. Journ. of Physiol.,' vol. 20, pp. 127—169, 1907.

‡ Walker, 'Introduction to Physical Chemistry,' 4th ed., p. 205, 1907.

Faraday showed, more than 50 years ago,* that the ruby-red solutions of gold which he prepared by reduction of gold chloride were suspensions of minute particles of metallic gold. He also noticed that the colour of the solution became blue under the action of sodium chloride in dilute solution, and was precipitated by stronger solutions. Both these effects were absent if a small amount of "jelly" had previously been added to the gold solution. This latter "protecting" action of "stable" colloids is now well known, forming the basis of Zsigmondy's "gold number" as a characteristic of proteins Congo-red behaves, as regards this protection from precipitation by electrolytes when a stable colloid is present, in the same way as the inorganic colloids. It may be noted, in passing, that this is a phenomenon usually ascribed to surface properties. The dye also is protected from adsorption by paper, under the influence of electrolytes, when a trace of gelatin is present.† It was, therefore, of interest to examine the influence of stable colloids on the reduction of osmotic pressure produced by electrolytes.

Since my experiments on adsorption above referred to indicated that this protective action was greater when the stable colloid had an electric charge of the same sign as that of the dye, or of opposite sign to that of the precipitating ion, I chose for the present experiments a dialysed solution of Grübler's serum-albumin, to which a trace of ammonium hydroxide was added in the first experiment. The solution in the osmometer contained about 0·18 per cent. of the dye and 0·25 per cent. of serum-albumin. When dialysed against distilled water, the osmotic pressure rose to 42 mm. of mercury. The water was then displaced by a decinormal solution of sodium chloride. To my surprise, the pressure fell to zero in about 14 hours. Moreover, as in the experiments without stable colloid, it was found that after 14 days' changes of distilled water the pressure could only be brought back to three-quarters of its original value. Measurements of the electrical conductivity of the water after interchange with the colloidal solution showed that the latter parted with its electrolytes very slowly. They were probably held in a state of adsorption by the protein as well as by the dye.

In another experiment I first tested the particular solution of serum-albumin used and found that in the proportion of 5 c.c. to 50 c.c. of one-thousandth normal congo-red solution, precipitation by one-hundredth normal calcium sulphate was prevented, although in the absence of the protein complete precipitation occurred. In order to be quite certain of adequate protection, I added 10 c.c. of the albumin solution to 50 c.c. of the dye and placed the mixture in a Schleicher and Schüll diffusion thimble of parchment-paper tied on to a glass tube fitted with a cork and a long narrow tube to act as manometer. This was placed in water contained in a large test-tube and then immersed in a thermostat at 30°·7 C. The osmotic pressure rose to 200 mm. of water in the course of 30 hours, being 94 per cent. of the theoretical value. Calcium sulphate in one-hundredth normal solution was then put into the outer tube instead of the water. In about 24 hours the pressure had fallen to 50 mm. and no further fall took place. In this case a certain protection was shown, since, without the albumin, the pressure would have gone down nearly to zero.

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* 'Phil. Trans.,' vol. 147, 1858 : As to the Nature of the Solutions, see pp. 160 and 172 ; Precipitation by Salt, p. 165 ; Protection from Action of Salt by "Jelly," p. 175.

† 'Biochem. Journ.,' vol. 1, p. 201, 1906.

The apparent disagreement between the results of osmotic pressure measurements and the naked eye appearances are, I think, to be explained in the following way. When the contents of the osmometer in the last experiment were poured into a glass vessel and observed carefully, it was obvious that, although no precipitation had taken place, the solution was distinctly more turbid than a similar one to which no calcium sulphate had been added. On examination under the ultra-microscope, the former was resolvable into a multitude of distinct, but not brilliant, particles; whereas solutions of congo-red itself, as already shown, are not resolvable. It is clear, therefore, that a certain degree of aggregation had in reality taken place, although the particles formed are much smaller than those formed when calcium sulphate acts upon congo-red in the absence of a protecting colloid. In this latter case, they are large enough to fall as a precipitate. The actual values of the osmotic pressure observed, 200 mm. and 50 mm., show that, under the conditions of this experiment, aggregates of four molecules are formed. It appears that, so far as congo-red is concerned, the mode of action of a stable colloid is to form, under the influence of an electrolyte, a colloidal complex with the dye, which complex, although consisting of several molecules and therefore, when formed, causing a large fall in the osmotic pressure of the solution, is yet in particles sufficiently minute not to fall as a precipitate. The reason why the aggregates formed are small is, no doubt, connected with the lowering of surface-tension caused by the protein. It is to be noted that, unless the solution of dye + albumin + electrolyte had been compared with a similar solution without the electrolyte, it would have been supposed that complete protection from the action of the electrolyte had been brought about, since no true precipitation occurs.

Results similar to the above have been obtained in the case of arsenious sulphide, aggregation without precipitation also occurs here. In order that the albumin shall be efficient as a protecting colloid it is necessary that it be of the same sign as regards its electric charge as the arsenious sulphide, that is, electro-negative; if electro-positive, it tends to aid the action of the electrolyte.

I am unable, at present, to state definitely whether the same considerations apply to the action of electrolytes and stable colloids on gold hydrosols, the "gold number" in fact. I cannot make out any difference in the appearances under the ultra-microscope of mixtures of gold hydrosols and serum-albumin, with and without calcium sulphate. To the naked eye, there is perhaps a slight tendency to a more purple colour in the case of the former, but nothing approaching the blue of the mixture of gold and electrolyte in the absence of the albumin. The ultra-microscope shows also that the particles in the blue solution are larger and less numerous than in the ruby-coloured ones. It is possible that complexes of gold particles and protective colloid, of the kind described by Zsigmondy,* may be formed without change of colour of the gold. The whole series of phenomena in these cases of protection are of much complexity and in need of further investigation.

It is somewhat remarkable that congo-red is the only dye which I have been able to make use of which shows this combination of non-diffusibility through parchment-paper with existence of single molecules in solution. The greater number pass through parchment-paper with more or less rapidity, although showing many colloidal properties, so that no permanent osmotic pressure can be obtained. Aniline-blue, with a molecular weight of 734, does not pass through. A solution containing 0·266 per cent. gave an osmotic pressure of 30·4 mm. Hg instead of the theoretical one of 68·8 mm. Hg, indicating a mean "solution-aggregate" of two molecules. It must be stated, however, that this solution was apparently not free from electrolyte, since, although when the experiment ceased no further permanent rise in osmotic pressure was produced by change of water, there was still a certain amount of "initial osmosis." The displaced water, also, showed a fairly high conductivity, 40 gemmhos, which did not diminish during the last five days. This outer solution was always stained faintly blue, due, as I shall show in another paper, to dissociation of the dye and permeability of the membrane to the products of this dissociation. In a similar way, the dialysate of congo-red is very faintly red. In the ultra-microscope the appearance of the solution removed from the osmometer was very like that of congo-red, as described above; a faint haze, with bright particles here and there, these bright points being much more numerous than in congo-red. I am inclined, therefore, to attribute the low osmotic pressure found to incomplete removal of electrolytes, the solution really being one of single molecules, but, owing to the electrolytes, a large number of these are aggregated to particles, the rest being single.

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* 'Zur Erkenntnis der Kolloide,' p. 116, Jena, 1905.

The free acid of congo-red, as already stated, forms a deep blue colloidal solution, with comparatively large particles, easily resolved bv the ultramicroscope. These particles are of a very nearly uniform size. In view of the debated question as to the source of osmotic pressure, it is of interest to see whether an unquestionable suspension, such as this is, has an appreciable osmotic pressure and, at the same time, to determine the number of particles present in unit volume. Since, as shown above, the salts of this acid have so considerable an osmotic pressure, while that of the particles of the acid would probably only be small, it is obviously of the greatest importance to ensure the absence of traces of alkali, whether in the water used or from glass vessels. The apparatus shown diagrammatically in fig. 2 was devised for this purpose. It will probably be found of use in the investigation of other colloids sensitive to traces of electrolytes, such as arsenious sulphide. All the tubes in contact with the fluids in the osmometer are of quartz and the containing vessel of glass is thickly coated with paraffin inside. The membrane is a Schleicher and Schüll parchment-paper thimble, as used by Waymouth Reid and by Hüfner. It is necessary to test these thimbles very carefully for holes and, for my purpose, to extract thoroughly before use with hydrochloric acid and distilled water to remove soluble salts. In future experiments it would perhaps be better to use membranes of collodion.

The distilled water must be carefully prepared. I found it best to distil tap water (New River) after adding potassium permanganate and sulphuric acid, and again after the addition of barium hydroxide. When essential to exclude carbon dioxide entirely, the water from the quartz condenser drops directly into the osmometer, as shown in the figure.

In an actual experiment with congo-red acid, the outer water was at first made acid with hydrochloric acid to ensure the absence of any salt of congo-red in the contents of the osmometer. The solution in the inside had been dialysed for a long time against distilled water. The concentration at the end of the experiment was 0·465 per cent. of the free acid. At the beginning of the experiment no measurable osmotic pressure was obtained; the acid was in the form of a precipitate, due to the presence of hydrochloric acid. Distilled water, from potassium permanganate and sulphuric acid, was then allowed to flow from the condenser, gradually displacing the hydrochloric acid. It is interesting to note that the osmotic pressure rose distinctly, while the outflowing water still gave reactions with silver nitrate and with

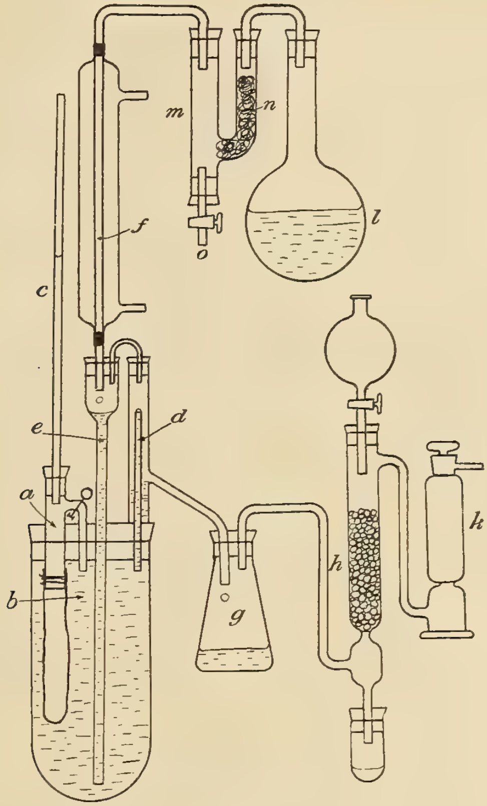

Fig. 2.

a, Tube on to which the parchment-paper thimble is tied. This tube has a side branch, connected by a short piece of rubber tubing to *b*, and thus, when the clip on the tubing is released, the inner and outer chambers are put in connection. This is of importance to control the zero point.
c, Narrow bore tube, serving as manometer. *d*, Outlet tube from outer chamber.
e, Tube conducting water from the quartz condenser, *f*.
g, Flask to collect the outflowing water, apart from contact with the atmosphere.
h, Tower containing dilute sulphuric acid. *k*, Soda-lime tower.
l, Flask for boiling water. *m*, Trap to collect spray. *n*, Glass wool.
o, Outlet for water condensed in *m*.

The containing vessel is thickly coated with paraffin and kept in a thermostat. The tubes *a*, *c*, *d*, *e*, and *f* are of quartz.

Günzberg's test, and, in fact, the pressure had risen to 60 mm. of water when 60 c.c. of the water neutralised 1·4 c.c. of decinormal ammonia. The distilled water, containing carbonic acid but no basic substance, was run in at intervals of 24 hours until no further rise in the osmotic pressure took place. The value attained was 91 mm. of water, or 6·8 mm. Hg at 30°·9 C. There seems no possibility whatever of the presence of a salt of the dye in the above conditions. In any case, there was a fairly large pressure when free hydrochloric acid was detected in the outer fluid. A further reason for denying the presence of alkaline bodies in the water used is that, if this had been so, the pressure would have continued to rise as long as fresh water was run in, until finally the great pressure of the salts of congo-red would have been reached and the contents of the osmometer converted entirely into the salt.

The conclusion must be that a definite osmotic pressure can be exerted by a solution consisting of an undoubted suspension of particles, resolvable under the ultra-microscope. When water distilled from over barium hydroxide was run in, there was not much further rise in the osmotic pressure, the maximum being only a few millimetres higher. It appears, therefore, that this colloid is not particularly sensitive to traces of free acid.

If now we proceed to calculate, from the concentration of such a colloidal solution as that of the above experiment, what the osmotic pressure should be if the dye were present in single molecules, we find that it is 20 times that actually found. Assuming for the present that the kinetic theory of the osmotic pressure of colloids is correct, this means that the average number of molecules forming a colloidal particle of the free acid of congo-red is 20.

This being so, and the separate particles being easily seen in the ultra-microscope, it seemed to be a point of interest to attempt to estimate the dimensions of the particles in the manner described by Siedentopf and Zsigmondy.* For this purpose a part of the contents of the osmometer at the end of the experiment was diluted 1300 times, so that the particles might be sufficiently far apart to be counted. This counting was somewhat difficult, owing to the rapid movement of the particles. The mean of a number of determinations was between 8 and 9 in a volume of 56×10^{-6} cubic millimetre. The undiluted solution, therefore, contained 2×10^{11} in 1 c.c. There is, however, a possibility not to be lost sight of. As I shall show in a later paper, this acid of congo-red appears to be very slightly soluble in water at 100°, in true solution, ionised. Although the amount dissolved at room temperature is infinitesimal, it may be sufficient to vitiate conclusions drawn from solutions necessarily very dilute. Accordingly, I have made determinations, similar to the above, with solutions of one and a-half and of twice the dilution of that one. The three values are placed together in the table for comparison. It will be seen that the values are, within the limits of error, proportional to the dilution.

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* 'Drude's Ann.,' vol. 10, pp. 17, 21, and 22, 1903.

Dilution (= number of litres containing 1 gramme).	Number of particles in 1 c.c.
280	$14—16 \times 10^7$
420	$10 \cdot 7—12 \cdot 5 \times 10^7$
560	$8 \cdot 9 \times 10^7$

Now, the total weight present in 1 c.c. of the original solution is 4·65 milligrammes, so that the weight of each particle is

$$\frac{4 \cdot 65}{2 \times 10^{11}} = 2 \cdot 3 \times 10^{-11} \text{ milligramme.}$$

Further, the specific gravity of the solid acid is 1·46, determined by weighing under toluene in a pyknometer.* Hence, the diameter of each particle is 310 $\mu\mu$.

We may, perhaps, go even further still. According to the osmotic pressure measurements and assuming the kinetic origin of this pressure, each particle contains on the average some 20 molecules; so that, if this theory be correct, we ought to be able to obtain an approximate value for the molecular dimensions of this body. When calculated from the data given, the weight of a molecule of congo-red acid comes out to be

$$1 \cdot 16 \times 10^{-12} \text{ milligramme,}$$

or nearly 10^9 times that of hydrogen.† And the diameter

$$111 \; \mu\mu.$$

The molecular weight being 652·372, the number of molecules contained in 1 gramme-molecule comes out as

$$\frac{652 \cdot 372}{1 \cdot 16 \times 10^{-15}} = 5 \cdot 6 \times 10^{18}.$$

The number of molecules in a gramme-molecule of a perfect gas is usually estimated at about

$$6 \times 10^{23}.‡$$

Considering the many sources of error, the result obtained for the molecular dimensions of our colloid does not seem very far out. This being so, the hypothesis of the kinetic origin of osmotic pressure is, so far, supported.

The chief difficulty in the estimation of the number of particles under the ultra-microscope is, in the case before us, the lively movements which they manifest. In order to stop this movement, I mixed a part of the diluted solution, as used for the previous measurements, with an equal volume of a 2-per-cent. solution of gelatin, warmed just sufficiently to liquefy it. This method was used by J. Duclaux,* in his investigations of ferric hydroxide. Although the gelatin used by me had been soaked in repeated changes of toluene-water, it retained a certain amount of its adsorbed electrolytes; so that on adding the blue colloid to it, a distinct change of colour towards purple resulted. Although the particles were seen to be immobilised, it did not seem worth while to proceed further with the laborious determinations, since the change of colour indicated a change in the colloid.

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* Ostwald-Luther, 'Phys.-Chem. Mess.,' 2te Aufl., p. 147, 1902.

† Walker, 'Introd. to Phys. Chem.,' 4th ed., p. 217.

‡ See Perrin, 'Comptes Rendus,' vol. 147, p. 531, 1908.

The determinations of molecular dimensions given above are intended to show the possibilities of the method. The exact numerical data are, no doubt, capable of correction when a more satisfactory means of immobilising the particles has been found. The values obtained appear high, even for a molecule containing 70 atoms, such as the one in question. From Zsigmondy's observations with colloidal gold it would seem that particles of these dimensions should be resolved by the ultra-microscope. It is true that the impression given to the observer is that the solutions of congo-red are just on the limit. Moreover, the fact that molecules of congo-red are unable to pass through parchment-paper shows that they far exceed in dimensions those of crystalloid bodies.†

Further experiments are in progress, as also others in the manner of those of Perrin‡ with suspensions of gamboge. It would be premature to draw conclusions from the results of the preceding pages as to whether the particles as wholes are responsible for the osmotic pressure, or whether only a part of each one, such as adsorbed ions, alone is active in this respect. So much may be said, that my observations speak decidedly in favour of the kinetic theory of the osmotic pressure of colloids. According to this theory the "Brownian movement" of the particles corresponds to the molecular movement assumed in the kinetic theory of gases.

Important recent confirmation of this view is to be found in the experiments of Perrin already alluded to, which show that the kinetic energy of a colloidal particle is identical with that of a molecule. This observer shows that, if we take the number of molecules contained in one gramme-molecule of a perfect gas to be

$$6 \text{ or } 7 \times 10^{23},$$

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* 'Comptes Rendus,' vol. 147, pp. 131—138, 1908.

† As regards size of pores in parchment-paper, see Bechhold, 'Zeit. f. Phys. Chem.,' vol. 64, pp. 328—342, 1908.

‡ 'Comptes Rendus,' vol. 146, pp. 967—970, and vol. 147, pp. 530—532, 1908.

as given by the kinetic theory, the osmotic pressure of a solution containing n molecules per unit volume is

$$n \times 40 \times 10^{-15} \text{ atmos.}$$

When deduced from the rate of fall of the particles in a gamboge suspension, assuming Stokes' formula to apply, and taking n to refer to particles, the osmotic pressure works out to be

$$n \times 36 \times 10^{-15} \text{ atmos.}$$

When deduced from the distribution of particles in a vertical column, after attainment of equilibrium, the formula becomes

$$n \times 42 \times 10^{-15} \text{ atmos.}$$

From my observations, determining the concentration of particles by direct enumeration under the ultra-microscope, the formula becomes

$$n \times 44 \times 10^{-15} \text{ atmos.}$$

Such close approximations to the theoretical value must be more than mere coincidence.

Ramsay and Senter* also concluded, from experiments on the density of arsenious sulphide solution taken by different methods, that the particles behave as if in true solution.

On the other hand, it is evident that my experiments lend no support to the theory according to which the osmotic pressure of a colloidal solution is due, in some way not very clear, to ions associated with the colloidal particles. It is difficult to understand how these ions can still exert their osmotic pressure when forming part of a complex system, which must move and act as a whole. This much may be said, congo-red gives an osmotic pressure which is at its highest when foreign electrolytes are most effectively excluded. This must be understood as in no way excluding, as the ultimate source of the negative charge, electrolytic dissociation of the colloid itself.

It is very doubtful whether electrolytes in the state of adsorption are ionised at all. Ruer† finds that the chlorine present in colloidal zirconium hydroxide gives no reaction with silver nitrate. Similarly in the case of ferric hydroxide, the chlorine can only be detected after destruction of the colloid by nitric acid.

The general conclusion to be drawn is, I think, that whether a body present in solution be in the form of particles, molecules or ions, each of these acts as an individual and equivalent element in the production of osmotic pressure.

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* B. A. Reports, 1901. In 1905 ('Journ. Phys. Chem.,' vol. 9, p. 319) Senter also made the suggestion that Brownian movement in colloids is equivalent to molecular movement in true solutions.

† 'Zeits. f. Anor . Chem.,' vol. 43, pp. 83—93, 1905.

Summary.

Congo-red, although a colloid in the sense of not being diffusible through parchment-paper and exhibiting certain other colloidal properties, has an osmotic pressure equal to that which would be given if it were present in true solution in single molecules.

The solutions are not resolvable into particles by the ultra-microscope.

The theoretical osmotic pressure is only to be obtained in the complete absence of extraneous electrolytes. Even the carbonic acid present in ordinary distilled water is sufficient to cause a marked fall in the osmotic pressure.

The manner in which electrolytes produce this fall is by causing aggregation of molecules to particles. This is the same whether acid, alkali, or neutral salt be in question.

The action of a stable colloid in protecting against the effect of electrolytes is shown to consist, in the cases of congo-red and arsenious sulphide, in the production of minute aggregates, which, although causing fall in osmotic pressure by diminution of effective concentration, are not of sufficient size to precipitate. Hence the protective power can only be regarded as a limited one, due probably to the formation of complex colloids.

The free acid of congo-red forms a blue colloidal solution when dialysed. This is easily resolvable under the ultra-microscope, but gives a definite and measurable, though small, osmotic pressure, about 14 mm. Hg for a 1-per-cent. solution. Assuming the kinetic theory to be correct, this means that the aggregates contain, on an average, 20 molecules.

Estimation of molecular dimensions are given on the basis of enumeration of the number of particles in unit volume by means of the ultra-microscope. The values found are considerably larger than the accepted ones for water, etc.

The whole of the results are capable of explanation on the assumption that colloidal particles possess the kinetic energy of molecules, but do not lend support to any view which postulates the necessary presence of foreign electrolytes.

(The expenses were defrayed by a grant from the Government Grant Committee of the Royal Society.)

Some Effects of Nitrogen-fixing Bacteria on the Growth of Non-Leguminous Plants.

By W. B. Bottomley, M.A., Professor of Botany in King's College, London.

(Communicated by Prof. J. Reynolds Green, F.R.S. Received April 20,—Read May 6, 1909.)

During the course of an investigation on "The Cross-inoculation of the nodule-forming bacteria from Leguminous and non-Leguminous plants,"* it was noticed that in all the bacterial cultures prepared from the algal zone of the root-tubercles of cycads taken from below the surface of the soil, *Pseudomonas radicicola* was associated with a species of *Azotobacter*.

In order to determine to what extent, if any, this association gave an increased power of assimilating free nitrogen, the two forms were obtained as pure cultures by successive platings on a medium composed of maltose 20 grammes, monobasic potassium phosphate 0·5 gramme, sodium chloride 0·5 gramme, calcium carbonate 0·5 gramme, ferrous sulphate 0·1 gramme, agar 15 grammes, and distilled water 1000 c.c. Separate cultures of each and a mixed culture were then grown in fluid media in duplicate 500 c.c. Erlenmeyer flasks containing 250 c.c. of the above medium, omitting the agar but adding 10 grammes of mannite. Control flasks were kept side by side with the inoculated flasks.

All the flasks were incubated at 24° C. for 15 days, care being taken to renew the air in the flasks at intervals, then a nitrogen determination was made of the contents of each flask. The results of these analyses gave the following averages:—

Control	0·48 milligramme N per 100 c.c.
Pseudomonas alone............	0·91 ,, ,, ,,
Pseudomonas + *Azotobacter*...	1·24 ,, ,, ,,

Hence *Pseudomonas* and *Azotobacter* together make a powerful combination for the fixation of free nitrogen.

The bacteria in the root-tubercles of cycads appear to live imbedded in the slime they produce *outside* the cortical cells in the open spaces of the so-called algal zone. The cortical cells which project into this zone presumably absorb the nitrogenous products of the bacterial activity, and thus the cycad is benefited. If, therefore, this combination of bacteria *outside* the cortical cells is a direct benefit to the cycas plant, the possibility presented itself that a mixed culture of *Pseudomonas* and *Azotobacter* applied directly to the roots of other non-leguminous plants might benefit their growth also.

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* Report British Association, 1907.

The first experiments were made on oats (*Avena sativa*). Four 5-inch pots were filled with sand freed as far as possible from organic matter and nitrates, and given a sufficient dressing of phosphates, potash, and lime. Twenty oat seeds were planted in each pot, and as soon as the young plants were about 1 inch high two of the pots were watered with a mixed culture of *Pseudomonas* and *Azotobacter* grown in the previously mentioned medium. The plants were watered regularly with distilled water and allowed to grow until the untreated plants exhibited signs of drooping. Each plant was then carefully air dried and weighed, with the following result:—

Untreated, average weight per plant	0·42 gramme
Treated	0·74 „

an increase of 0·32 gramme, or 76 per cent.

In 1908, field experiments were made on barley. Two plots, each having an area of 484 square yards, were planted, one with seed moistened with the mixed culture, the other with seed untreated. The land was very poor and low in organic nitrogen, having carried oats in 1906 and barley in 1907. The yield of grain per plot at harvest was:—

Untreated...........	608 lb.
Treated..............	691 „

an increase of 83 lb., or 13·6 per cent.

On the same farm a strip of land was sown with treated seed through the centre of a 34-acre barley field. It was found impossible at harvest to keep the yield of treated seed separate from the rest, but samples of grain from the treated and untreated parts were taken and analysed, and it was found that the barley from the treated seed had the higher nitrogen-content.

	Milligrammes of N per cent.	Weight of 1000 corns.	Milligramme N per corn.
Untreated......	1·55	48·5	0·75
Treated	1·76	49·5	0·87

Experiments on hyacinths (*Galtonia candicans*) grown in sandy soil. The soil was dressed with lime in August, 1907, and remained fallow until April, 1908, when it was manured with cow manure—10 tons to the acre. Bulbs of equal size were then planted, 250 in each bed, a path of 14 inches dividing the two beds. The treated bulbs were twice watered with mixed culture solution, once in May and once in June, the control bed being watered with pure water at the same time. The difference between the treated and untreated bulbs was very noticeable during growth, the treated being more vigorous. The treated bulbs, when lifted, were noticeably larger than the untreated. After the bulbs were dry they were carefully weighed, and yielded

Untreated.........	69 lb. 3 oz.
Treated	82 „ 1½ „

an increase of 12 lb. 14½ oz., or 18·6 per cent.

Experiments on parsnips grown in ordinary garden soil. In January, 1908, the ground was deeply dug and given a medium dressing of London dung followed by a dressing of powdered chalk. The seeds were sown early in February in rows running north and south. A fortnight after the north half of each row was watered with a mixed culture of *Pseudomonas* and *Azotobacter*. In January, 1909, the roots were harvested, *every* root being weighed, with the following results:—

Untreated......	68 roots	22 lb. 14 oz.	5·38 oz., average per root
Treated	65 „	26 „ 10 „	6·55 „ „

an increase of 21·7 per cent.

For the fixation of free nitrogen in laboratory cultures of *Pseudomonas* and *Azotobacter* the presence of carbonate of lime in the medium is necessary. In all the above experiments care was taken that a sufficiency of carbonate of lime was present in the soil to enable the bacteria to do their work effectively.

The Intracranial Vascular System of Sphenodon.

By Arthur Dendy, F.R.S.

(Received March 30,—Read April 22, 1909.)

(Abstract.)

This memoir contains a detailed description, with illustrations, of the intracranial blood-vessels of the Tuatara, of which no account has hitherto been published. The description is believed to be more complete than any hitherto given for any reptile, and a considerable number of vessels are described which have not hitherto been noted in Lacertilia. This comparative completeness of detail is largely due to the employment of a special method of investigation. By this method the entire contents of the cranial cavity are fixed and hardened *in situ,* and are then in excellent condition either for dissection or for histological purposes. The brain does not occupy nearly the whole of the cranial cavity, there being a very large subdural space (especially above the brain), across which many of the blood-vessels run, together with delicate strands of connective tissue which connect the dura mater with the pia. The eyeballs are removed and an incision is made on each side in the cartilaginous wall which separates the cranial cavity from the orbit. Acetic bichromate of potash (made up according to the formula given by Bolles Lee) is injected into the cranial cavity through these incisions, and the entire animal, after opening the body cavity, is suspended in a large volume of the same fluid for about five days, and then graded up to 70 per cent. alcohol. When the cranial cavity is now opened up the cerebral vessels are seen with extraordinary distinctness, although they have not been artificially injected.

Further details were made out by means of serial sections, both transverse and longitudinal, and both of the adult and of advanced embryos (Stage S). In most respects the arrangement of the intracranial blood-vessels agrees with that found in the Lacertilia, so far as these have been investigated, but there is an important difference in the fact that the posterior cephalic vein leaves the cranial cavity through the foramen jugulare and not through the foramen magnum, while a slightly more primitive condition is shown in the less complete union of the right and left halves of the basilar artery. *Sphenodon* makes some approach to the condition of the *Chelonia* in this latter respect, but differs conspicuously from this group in the fact that the circle of Willis is not completed anteriorly, as well as in the fact that no branch of the posterior cephalic

vein leaves the cranial cavity through the foramen magnum. A very characteristic feature of *Sphenodon* is the development of large transverse sinuses resembling those of the crocodile, but these communicate with the extracranial vascular system in quite a different manner from that described by Rathke in the latter animal.

The Variations in the Pressure and Composition of the Blood in Cholera; and their Bearing on the Success of Hypertonic Saline Transfusion in its Treatment.

By Leonard Rogers, M.D., F.R.C.P., F.R.C.S., I.M.S., Professor of Pathology, Calcutta.

(Communicated by Sir T. Lauder Brunton, Bart., F.R.S. Received December 4, 1908,—Read January 28, 1909.)

During the quarter of a century which has elapsed since the discovery by Prof. Koch of the comma bacillus of cholera, research work has been almost confined to the bacteriology of the subject. Unfortunately, with the exception of M. Haffkine's prophylactic inoculations, which are now very little used even in India, this line of work has done little or nothing to help the practitioner who is confronted with the treatment of this terrible disease. No powerful antitoxic serum of practical value has been produced, and even if such should still be obtained, many patients come under treatment in such a state of collapse that no medicine can be absorbed, even if retained.

The old controversy between the evacuant and conservative methods of treatment has long since ended in the practically universal adoption of the latter, although as late as 1866 Dr. George Johnson advocated castor oil, denying that there was any relationship between the amount of fluid lost from the body and the mortality, while he strenuously opposed the use of intravenous saline injections to replace it. There is still much difference of opinion about the latter treatment, for although all who have used transfusions testify to the remarkable immediate improvement in the pulse and general condition, yet this is commonly of such brief duration that many think it only serves to needlessly prolong the agony of the patient, so that of recent years it has been only exceptionally resorted to in India.

For a long time I have been investigating the blood changes in cholera (1), in the hope of finding some indication for a more rational and successful line of treatment, than the mere administration of more or less useful drugs, into a gastro-intestinal tract, which is too congested to retain any appreciable powers of absorption. The great loss of fluid from the blood was shown by marked increase of the number of red corpuscles per cubic millimetre, which sometimes rose from 5,000,000 to over 8,000,000, while, like others, I found leucocytosis to be constantly present, and when of a marked degree to be of bad prognostic value. Differential counts showed a great decrease of the lymphocytes, corresponding with their accumulation in the lymphoid tissue of the alimentary tract, while the large mononuclears were markedly increased, both relatively and absolutely, this change being in proportion to the mortality, indicating its probable relationship to some specific toxin of the disease. These changes were often of great value in the difficult diagnosis of cholera from ptomaine poisoning, as I have not found them in the latter affection.

More recently I have studied the blood-pressure in cholera as an indication for the necessity of transfusion and the quantities to be injected. My first experiments in this line were carried out in conjunction with Captain J. W. D. Megaw, I.M.S. (2), who was in charge of the cholera patients at the Calcutta Medical College, normal saline solution (1 drachm to the pint) being used in accordance with the general custom. The patients were nearly all natives of India, whose normal blood-pressure averages only about 100 mm., being thus considerably lower than that of Europeans. In cholera, the pressure was rarely over 50 mm. on the admission of the patients, while in many it was too low to be estimated in the radial artery, owing to absence of any pulse at the wrist. We found that 1 pint of saline solution injected into a vein usually had very little effect in raising it, but a second pint generally increased it to 90 mm. or more. The immediate effects of this treatment were little short of marvellous. The terrible restlessness and cramps disappeared, and the worn out patient often fell asleep before its completion. The pulse was fully restored, and the blueness and coldness were replaced by the normal pink hue of the lips and nails and warmth of the extremities. A few improved steadily from that time, but only too frequently within 2 to 12 hours the copious rice water stools and vomiting recurred, and the patient relapsed into as bad a condition as before, with complete loss of the improvement in the blood-pressure. Repeated transfusions usually failed to save such patients, and although the mortality did show some reduction during the year Captain Megaw continued them, yet the disappointments were so many that the method soon came to be adopted only in a few desperate cases, and was eventually almost entirely abandoned once more, as it had been by so many earlier enthusiasts.

On thinking over the causes of this comparative failure, the following possible explanation occurred to me. The great loss of fluid through the stomach and bowels produces a concentration of the blood, which might be expected to increase the proportion of salts it contains, and therefore present a greater osmotic resistance to further draining off of fluid through the damaged intestinal mucous membrane. Thus, a conservative process, tending to check the diarrhœa, would come into action, which would be interfered with by the injection into the blood stream of large quantities of normal salt solution of lower salt content than the now concentrated hypertonic blood-serum. The drain through the bowel would therefore be restarted, and the restored fluid and blood-pressure rapidly lost again, as is so commonly seen in actual practice. If this view is correct, the indication would be to inject hypertonic salt solutions, so as to supply sufficient fluid to restore the circulation, and at the same time maintain the conservative beneficial hypertonicity of the blood, which would tend to carry more fluid into the circulation, instead of removing it through the damaged bowel wall. In 1907 I had an opportunity of discussing this point with both Sir Lauder Brunton, Bart., F.R.S., and Prof. Buckmaster, who were of the opinion that moderately hypertonic salt solutions might safely be injected intravenously.

The unusually great prevalence of cholera during the first half of 1908 in Calcutta furnished abundant opportunities for testing the value of intravenous transfusions of hypertonic salt solutions, for a trial of which I am greatly indebted to Captain Maxwell Mackelvie, I.M.S., who had in the meantime succeeded to the charge of the cholera wards. At first, 0·95-per-cent. sodium chloride solution was tried, and, as the results appeared to be distinctly more favourable than with the previously used 0·65-per-cent. one, it was soon raised to 1·35, or 2 drachms to the pint. The quantities injected were also raised by Captain Mackelvie to 3 or 4 pints at a time. In a note (3) published in the 'Indian Medical Gazette' in May, 1908, we recorded the results in 72 consecutive cases, in which transfusion with the above strength was used in all who required it, with a mortality of only 27·8 per cent., as compared with an average during the five years preceding the revival of transfusion at the hospital of 61·2 per cent. Moreover, at a neighbouring hospital, during the same period as our cases were treated, the mortality was just twice as great, intravenous injections not being used there, although their previous mortality during a series of years was practically the same as at the Medical College Hospital, namely, 63 against 61 per cent. At the present time our cases number 175, with a death-rate of 33 per cent., including moribund and complicated ones, which is but a very little over half that of the pre-transfusion period, although the mortality is always exceptionally high in

Table of Blood Changes in Cholera.

	1	2	3	4	5	6	7	8	9	10	
	Percentage of serum in the blood.	Percentage loss of serum from the blood.	Total loss of fluid from the blood.	Pints of saline intravenously.	Percentage of serum after transfusion.	Blood-pressure.		Strength of NaCl injected.	Percentage of chlorides in blood.		Remarks.
						Before transfusion.	After transfusion.		Before transfusion.	After transfusion.	
					I. Fatal Cases examined in Acute Stage.						
			ozs.					per cent.			
1	30	65	43	4	66	40	80	1·35	0·73	0·89	2nd transfusion.
2	35	55	36	3	65	50	80	1·35	—	—	3rd transfusion.
3	38	51	33½	4	59	Nil	100	1·35			
4	21	78	51½	3	—	,,	115	1·35			
5	11	90	59½	4	50	,,	110	1·35	0·67	0·88	2nd transfusion.
6	31	64	42	4	69	30	100	1·35	1·00	1·09	Very old man.
7	39	47	31	4	—	—	—	1·35	0·75		
Averages	—	64	42·3	—	—	—	—	—	0·79	0·95	
					II. Cases of Recovery after Transfusion.						
8	42	40	26½	2	56	—	110	1·35	0·74	1·04	
9	34	58	38½	3½	70	55	105	1·35	0·90	1·27	
10	29	71	47	4	62	35	100	1·35	—	0·80	Severe, 2nd transfusion.
11	45	33	21½	3½	—	55	110	1·35	0·74		
12	43	37	24	4	59	—	—	1·35	0·94	1·05	
13	38	51	33½	4	—	—	—	1·35	1·05	1·02	
14	32	62	41	4	56	60	110	1 35	1·22	1·25	2nd transfusion.
15	36	55	36	?	—	—	—	—	0·98	1·21	
16	32	62	41	4	64	—	—	—	0·74	0·86	Severe.
17	—	—	—	?	—	—	—	—	0·69	0·99	
18	—	—	—	4	—	—	—	—	0·68	1·00	
19	—	—	—	3	—	—	—	—	1·22	1·25	
Averages	—	52	34	—	—						
					III. Cases of Recovery without Transfusion.						
20	43	37	24								
21	42	40	26½	—	—	55					
22	47	27	18	—	—	60	—	—	1·06		
23	49	22	14½	—	—	90	—	—	0·81		
24	38	51	33½	—	—	80	—	—	0·90		
Averages	—	35	23·3	—	—	—	—	—	0·90	1·07	

such epidemic years as the present one, and the patients were frequently too numerous to allow of as many being transfused as we should have liked. Very favourable reports have also reached me from two other medical officers who have tried hypertonic solutions in cholera.

During the last few months I have made a number of observations on the blood changes before and after transfusion in cholera, which appear to furnish a rational basis for the regular use of large hypertonic transfusions in the treatment of the disease, and propose to deal with them in the present communication. The data have all been tabulated for convenience of reference.

The Relationship of the Amount of Fluid lost from the Blood in Cholera to the Degree of Collapse and the Death-rate.

As has been pointed out by Macnamara, Wall, and others, the amount of the watery evacuations in cholera will not furnish accurate data for estimating the degree to which the blood is drained of fluid, for the very rapid escape of 2 or 3 pints will have a far greater effect on the circulation than much more considerable loss spread over a longer time. Thus, in the so-called cholera sicca, in which death takes place without actual diarrhœa, the small intestines always contain several pints of liquid, the rapid draining of which from the blood has caused fatal circulatory failure. In order to ascertain the real loss of fluid from the blood in cholera, and the effects of various quantities of intravenous saline injections required to replace it, I have taken blood immediately before and after transfusion, defibrinated it at once, and measured the relative volumes of corpuscles and serum by centrifuging in the hæmocrite. As the red corpuscles are not lost to the circulation, but only the fluids of the blood, the reduction in the volume of serum can be readily calculated, and the actual loss of fluid from the serum of the blood estimated from the total volume of the blood in the body. According to some observations on healthy Bengalis by Captain McCay, I.M.S., the average proportions of corpuscles and serum respectively were 45 and 55 per cent., showing a slightly smaller proportion of corpuscles than in Europeans. Assuming that the red corpuscles are not lost from the circulation, the diminution in the serum can be calculated by the following formula from the hæmocrite figures. As the percentage volume of corpuscles found is to the normal value (45 per cent.), so is the volume of serum found to X. For example, if in a case of cholera the volume of corpuscles is 71 per cent. and of serum 29 per cent., then as

$$71 : 45 :: 29 : X, \quad \text{and} \quad X = 16.$$

That is, out of 55 original volumes of serum in the normal native blood only 16 remain, so the loss is 39 out of 55 volumes, or 71 per cent. of the total serum of the blood. In this way the figures given in column 2 have been arrived at. Further, taking the total circulating blood as one-thirteenth of the body weight (the exact amount is still disputed, but as my figures have but a relative value, this is immaterial in the present instance), I estimate the amount of blood in the body of a native of average weight as 6 pints, or 120 ounces. The percentage of serum being 55, this would give 66 ounces of blood serum in the circulation. The percentage loss in a given case having been calculated as above, the absolute loss in ounces is easily obtained, and the figures are given in column 3 of the table.

The cases have been subdivided, in accordance with the severity of the cases, into the three following classes. Firstly, those examined in the acute stage of the disease, which proved fatal from its direct effects. (Four cases in which the patients recovered from the collapse stage to die of late complications have been omitted, as the deaths were due to such affections as dysentery and parotid abscess, which afford no indication of the original severity of the cholera.) Secondly, those who showed such well-marked collapse as to require intravenous transfusion, but ultimately made a good recovery. Thirdly, milder, though well-marked, cases of cholera, in which transfusion was not necessary. The disease in most of the cases was confirmed bacteriologically, although the diagnosis was left to the physician in charge, so as to be strictly comparable with earlier series.

The average loss of fluid from the blood in each class is given in the table, and clearly demonstrates that the urgency and fatality of the cases was in direct proportion to the diminution in the volume of the blood-serum. Thus, among those in which the disease proved directly fatal, the loss of serum averaged no less than 64 per cent., or almost two-thirds, the total loss being 42 out of 66 ounces. In the second class of recoveries after transfusion there was a loss of 52 per cent. of the serum, or 34 ounces, while in the third series of recoveries without transfusion the losses averaged only 35 per cent., or 23·3 ounces.

The Specific Gravity of the Blood in Cholera.—The above observations furnish accurate estimations of the concentration of the blood due to loss of fluid in cholera, and demonstrate a definite relationship between its degree and the severity of the disease, while they indicate the necessity of replacing the loss in severe cases by some form of transfusion. For clinical purposes, however, some simpler method of rapidly ascertaining the required information is essential, especially in Indian hospitals far removed from laboratory conveniences. An easy, and sufficiently accurate, way is to estimate the specific gravity of the blood by the chloroform and benzene method, or more conveniently in a hot climate by Lloyd Jones' plan of using a series of small bottles containing solutions of glycerine in water of different specific gravities, into several of which a drop of blood can be placed until the one in which it neither sinks nor rises is found. I have made a number of observations in this manner, and find that in bad cases of cholera the specific gravity usually rises over 1070, while in those which did not require transfusion it only reached up to about 1065, so that a clinically valuable approximate estimation of the concentration of the blood in cholera can thus be very rapidly obtained at the bedside. Moreover, this test can be repeated during transfusion so as to ascertain when the blood has been diluted down to its normal point, or better, a little below it, and the quantity of fluid injected can be so regulated.

The Quantity of Fluid required to replace the Loss in Cholera.

Columns 1 and 5 in the table show the percentage volume of serum in the blood before and after intravenous transfusion with 1·35 sodium chloride solution in the quantities indicated in column 4. The usual amount was 4 pints, and in several successful cases this quantity was repeated a second time. These are larger amounts than are usually recommended for intravenous use in cholera, but have given very good results. Moreover, column 5 of the table shows that they did not increase the volume of serum very materially above the normal level of 55 per cent., while any excess will pass rapidly into the greatly drained tissues, and some allowance must be made for further losses through the bowel before the disease completely subsides. In several cases the blood was found to have again become so concentrated by the following day as to necessitate a second injection, with ultimate recovery, so that the amounts used were certainly not excessive. At the same time there must be a limit to the quantities that can be rapidly administered intravenously at one time, and the fact that in several cases the volume of serum was raised to between 65 and 70 per cent. appears to indicate that 4 pints is sufficient in most cases. In the very exceptional concentration of the blood in case 5, however, even this amount failed to increase the serum even to the normal point, the specific gravity after the injection being also 1064, or considerably above the normal, and the patient ultimately died.

In the less severe types much fluid can be got into the system by rectal injections, which are of great value. Subcutaneous injections are often used, but act much more slowly than intravenous ones in raising the blood-pressure, and are very liable to be followed by abscess in the low state of vitality of the tissues in cholera. I have recently had a simple cannula constructed with circular end sharpened like a cork borer, which can be safely passed through the abdominal wall after incising the skin and fascia. By this means saline fluid can be rapidly run directly into the abdominal cavity, and it has been successfully used in a number of cholera cases in one of the Calcutta hospitals. It can also be carried out in a much shorter time than the more difficult operation of tying a cannula into the collapsed vein of a cholera patient, but it takes several hours to be absorbed, so is less efficient in urgent cases than the intravenous injections. The hypertonic solutions have been found to give better results than normal saline by all methods of administration.

The Loss of Chlorides from the Blood in Relation to Hypertonic Transfusions.

Edmund Parkes, in 1849, showed that the rice-water stools of cholera contain very little albumen, but from ½ to 1 per cent. of salts, so that for every hundred ounces of fluid evacuations from the bowel nearly an ounce of salt is lost from the blood and tissues. The main bulk of the salts consists of chlorides, which may be taken as a guide to the amount in the blood at any time. Some recent estimations made by me gave an average of 0·53 per cent. of chlorides in cholera stools. The vomited matter contains much less salt than the bowel discharges, apparently owing to the gastric mucous membrane being less involved in the disease processes than the intestinal. Different observers have obtained very varying results as regards the percentage of salts found in the blood in cholera, some maintaining that they are higher, and others lower, than normal, but the observations on this point in the very limited literature available in Calcutta were all made many years ago. I have, therefore, estimated by the silver nitrate method the amount of chlorides in the blood in a number of cases both before and after transfusion with the 1·35-per-cent. sodium chloride solution. The results are shown in columns 9 and 10 of the table.

Taking first cases 1—7, which were fatal in the acute stage, we must bear in mind that on the average two-thirds of the fluid had been lost from their blood. If only the water had been removed without any saline constituents, then the chlorides should have been present in three times the normal amounts. The percentage of salts in the blood in Bengalis has been found by Captain McCay to be somewhat higher than in Europeans, namely about 1 per cent., about 0·8 per cent. of which are chlorides. Yet in the fatal cholera cases, in spite of the great concentration of the blood, the chlorides were below the normal in four out of five, the single exception being a very old man who died of heart failure. Fully two-thirds of the total chlorides of the blood must, therefore, have been lost. In case 5, with only 0·67 per cent. of chlorides, the serum showed actual commencing hæmolysis, which completely disappeared when they had been raised to 0·88 by hypertonic transfusion, while similar results have been noted in a few other cases with very low salt content. Column 10 shows that a material rise in the percentage of chlorides in the blood was obtained by transfusion of 3 to 4 pints of 1·35-per-cent. sodium chloride solution, but the average amount after it was still only 0·95 per cent.

On turning to the recovering cases 8 to 24, we find the average percentage of chlorides in the blood before transfusion was 0·90, or considerably higher than in the fatal series, and the same remark applies to the figures obtained after transfusion, when the average was 1·07 per cent. Moreover, in cases 10 and 16, which showed a low amount of chlorides after the injection, the disease was very severe, and in one a second transfusion was necessary to save the patient. It will be observed that several of the cases, which recovered after the injections, showed very low chlorides in the blood at the first estimation, but when they were raised to about 1 per cent. or over by the hypertonic solutions, the patients almost invariably did well as far as the collapse stage of the disease was concerned, which is the most dangerous period in cholera.

Another striking feature was the far less tendency of the hypertonic solutions to restart the copious rice-water stools, which so commonly renders the use of normal salines of such very temporary value. It is not too much to say that at the Medical College Hospital, where cholera patients are usually brought in an advanced stage of collapse, the simple substitution of 2 drachms of salt to the pint instead of one, for transfusion, has so revolutionised the treatment, that, whereas formerly it was considered a matter for surprise when a severe case of cholera recovered, it is now a great disappointment when such a case is lost in the collapse stage.

The Coagulability of the Blood in Cholera.

A few observations on the clotting power of the blood have been made by Sir Almroth Wright's method. The results were very variable, the time being normal in some, slightly reduced in most, and very markedly in a few. It is noteworthy that in several of the worst cases, with a specially low percentage of chlorides, blood taken in glass tubes remained quite uncoagulated after several hours, and in one such case there were hæmorrhagic stools, found *post mortem* to be dependent on extensive petechial hæmorrhages in the cæcum. After transfusion with hypertonic solutions the blood in such cases clotted firmly and gave a clear serum. In view, however, of the frequency of reduction of the clotting power of the blood in cholera, I now add 3 grains of calcium chloride to each pint of salt solution, and have seen no hæmorrhagic stools in the few cases since treated.

Strength of the Hypertonic Solutions.

In a few cases, 1·65-per-cent. solution (2½ drachms to the pint) were used with good results, but as the 1·35 per cent. has proved successful in so many cases, and, moreover, suffices to raise the chlorides in the blood well above the normal, it is recommended for routine use. The higher strength may be reserved for second transfusions in very severe cases, or when the lower strength has not increased the percentage of chlorides in the blood to the desired point of 1 per cent. or over, and especially if watery diarrhœa continues. We have never seen any harm from running these solutions rapidly into a vein in cholera, 4 pints having frequently been administered in 20 minutes. The specific gravity of the 1·35 solution is 1006, and of 1·65 is 1008, a knowledge of which allows the strength of the solution to be rapidly verified.

Effects of Intravenous Saline Injections on the Blood-pressure.

The blood-pressures immediately before and after transfusions are shown in columns 6 and 7 of the table. Nil means that there was absolutely no pulse at the wrist, so it could not be recorded in the radial artery, which was the site of the other observations. It usually required about three pints to raise the blood-pressure to 100 mm., which is the normal for Hindus. Experience showed that the best results were obtained by continuing the injections until the pressure rose somewhat above that point, for which purpose 4 pints were generally necessary, and allow some reserve. In some of the worst cases it was impossible to get it above 80 mm., as in numbers 1 and 2 in the table, for it remained at that point in spite of another pint being run in. One instance was met with in which even 6½ pints failed to raise the pressure above 65 mm. In such there appears to be some vasomotor paralysis present, possibly due to absorption of albumoses through the damaged intestinal mucous membrane. Adrenalin forces up the pressure in them, but its effects are only very temporary, as I formerly found it to be in the vasomotor paralysis of viperine snake poisons (4). The blood-pressure, then, is a valuable guide to the amount of saline solution to be run in, a pressure slightly above the normal being aimed at; but if 4 or more pints have been given, and the last 20 ounces or so have produced no further improvement, it is useless to continue it at that time. Fortunately a degree of vasomotor paralysis which prevents the pressure being raised to 90 or 100 mm. in natives of India is rare, as such have always terminated fatally in my experience.

The Relationship of Blood-pressure to Post-choleraic Uræmia.

One result of tiding so many severe cases of cholera over the collapse stage by large hypertonic saline transfusions, is to accentuate the importance of uræmia in the later stages of the disease. This very serious complication is especially seen in two distinct classes of cases :—Firstly, in patients brought to hospital more than 48 hours after the onset of the symptoms, which not infrequently have been comparatively mild at the onset, and whose treatment has been neglected. Secondly, the other extreme of patients admitted early on account of the great severity of the affection, who have been supported by repeated transfusions to the stage of reaction, when it is found to be extremely difficult to restore the renal secretory activity, which has been in abeyance during the prolonged period of very low blood-pressure.

On cutting sections of the kidneys of patients who had died in the uræmic stage of cholera, I was much struck by the amount of effused blood in and around the convoluted and straight tubules and the tense state of the capsule enclosing the extremely congested organ : all suggesting an actual mechanical difficulty in the re-establishment of an efficient circulation through the organ. In order to test if this was the case or not, I tried perfusion of normal saline solution through the renal artery from different heights, so as to measure the actual pressure required to obtain a fairly full outflow from the renal veins. For this purpose I used both healthy kidneys, got from the bodies of patients dying of other diseases, and also several from those who had succumbed to the uræmia of cholera. In the former, a pressure equivalent to 20 or 30 mm. of mercury sufficed to obtain a good flow through the kidney circulation. On the other hand, in the cholera ones no flow at all was got under a pressure of about 60 mm., and then only drop by drop, and it was not until 90 or 100 mm. was reached that anything like a full stream was observed. In one experiment, subsequent slitting of the capsule of the organ reduced the pressure required by about 20 mm. In a case of cholera in which the patient died of late complication with empyema, after the secretion of the urine had been freely established a pressure equivalent to 30 mm. of mercury sufficed for the free perfusion of normal saline through the kidneys, showing that it was only in uræmic cases that obstruction of the renal circulation existed in such a marked degree.

Since the above observations were made, the blood-pressure has been carefully watched day by day after the termination of the collapse stage, and it has been found that the uræmic symptoms are more common in those whose blood-pressures do not rise above 100 mm. For example, two very severe cases of cholera were admitted to hospital at about the same time, both of whom received two saline transfusions of 4 pints each, and recovered from the collapse stage. On the fifth day both had passed little or no urine and showed definite uræmic symptoms. The blood-pressure of one was now only just 100 mm. and he died the following night of uræmia. That of the other was 120 mm., and he began to pass urine that day, and made a good recovery. Soon after another patient developed well-marked uræmia, his respirations being over 40 per minute and very laboured, while he was practically unconscious, and apparently in a hopeless state. As his blood-pressure was found to be only just 100 mm., adrenalin and digitalis were administered subcutaneously, and on the following morning his pressure was 110 mm., he had recovered consciousness, was passing urine freely, and got well from that time. Thus, the indications derived from the *post-mortem* kidney perfusion experiments have been borne out in practice, and it is clear that the blood-pressure is the most important factor to be attended to in the treatment of the deficient renal secretion, which ensues in so many severe cholera cases, after the danger of death from collapse has been averted by saline transfusions.

Conclusions.

1. In cholera there is a very definite relationship between the amount of fluid lost from the blood and the severity and mortality of the disease.

2. This loss is usually so great as to indicate saline transfusions to restore the circulation.

3. Injections of normal salt solutions are commonly of only very temporary benefit.

4. Hypertonic salt solutions (1·35-per-cent. sodium chloride, or 2 drachms to the pint) are much more effective, their use having reduced the mortality by about one-half.

5. A great loss of chlorides from the blood occurs in cholera, most marked in the worst cases. If the percentage of chloride is considerably raised by the intravenous injection of hypertonic salt solutions recovery usually ensues.

6. The development of uræmia in the reaction stage of cholera is associated with a comparatively low blood-pressure, measures to raise which are indicated for the prevention and treatment of this very serious complication.

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The Effect of Heat upon the Electrical State of Living Tissues.

By A. D. Waller, M.D., F.R.S.

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I. *Of Muscle.*

The question whether the sudden application of heat acts as a physiological stimulus to nerve and muscle naturally leads on to a study of the effect of local heat upon the electrical state of living tissues. I have done this (1) upon muscle, (2) upon nerve, and (3) upon the skin. As far as I know, the only tissue hitherto tested in this respect is muscle.*

Du Bois-Reymond first,† then Worm-Müller‡ in more detail, observed that a muscle dipping in an indifferent fluid that was gradually heated and led off to the galvanometer from the fluid and from the undipped muscle exhibited, with rise of temperature, positivity followed by negativity of the undipped portion. Hermann, in 1870,§ flatly contradicts this statement, but in the following year|| gives an account of experiments from which he concludes that warmer portions of living muscle are positive in relation to cooler portions, *i.e.* that differences of temperature in the muscle give rise to a special electromotive force (eine besondere elektromotorische Kraft). But the protocols of his experiments, especially when they are plotted as curves, are not very convincing. The question is one that requires to be carefully re-tested. None of the experiments quoted affords any conclusive proof that the influence of rise of temperature upon muscle currents were true physiological effects apart from physical (thermo-electric) changes.

The method employed in the present series of observations was as indicated in the following representative experiment.

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* I learn that Engelmann, in 1872, examined the influence of temperature upon skin-currents, obtaining with rise of temperature a negative variation of the normal current, 'Pflüger's Archiv,' vol. 6, p. 138.

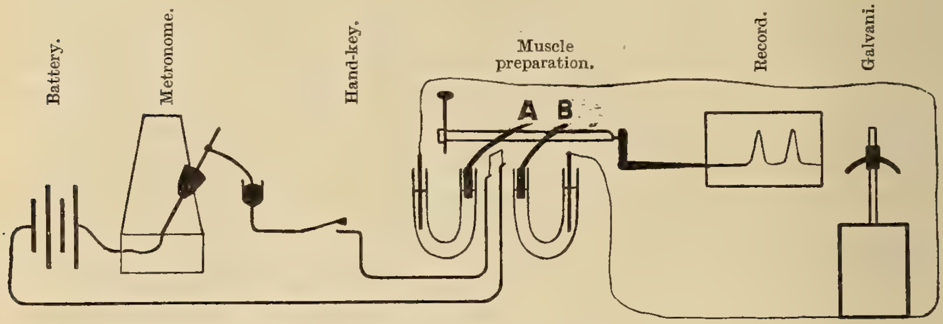
† Du Bois-Reymond, 'Untersuchungen über Thierische Elektricität,' vol. 2, p. 178; 'Gesammelte Abhandlungen,' etc., vol. 2, p. 202.

‡ 'Worm-Müller, 'Versuche über die Einflusse der Wärme auf die elektromotorischen Kräfte der Muskeln und Nerven,' Würzburg, 1868.

§ Hermann, "Versuche über den Verlauf der Stromentwicklung beim Absterben." 'Pflüger's Archiv,' vol. 3, p. 43, 1870: "Genau bei 40° . . . . entwickelt sich ein im lebenden Muskel aufsteigender Strom. Die absterbende Muskelsubstanz erlangt also ihre Negativität gegen die lebende in dem Momente der Erstarrung. . . . . Eine (nicht thermoelektrische) Positivität der erwarmten Substanz im Sinne Worm-Müller's existirt nicht."

|| Hermann, same title, 'Plüger's Archiv,' vol. 4, p. 163, 1871.

A sartorius muscle led off to the galvanometer in the usual way. A platinum wire adjusted transversely under one of the electrodes A or B of an accumulator, a metronome key and a hand-key K, all in the same circuit, so that the metronome gives regular brief periods of closure (when the hand-key is also closed) that raise the platinum wire to a red heat for a fraction of a second. The glowing wire heats the muscle locally at A by radiation and by the ascending current of heated air. The heat is graduated by bringing the wire nearer or farther from the muscle, or by varying the number of effective closures, or by varying a resistance in the accumulator circuit. It can be calculated if required. But the important condition to be secured is not so much an accurately known amount of heat as an accurately repeated application of a definite regular amount of heat. In these experiments the heat at each glow of the wire amounted to 0·08 calorie.



The movements of the muscle are recorded by a lever on the smoked cylinder or plate. The movements of the galvanometer mirror indicative of the electrical changes are at the same time recorded photographically.

Result:—

Moderate heat at B gives current in the muscle from A to B.

Excessive heat at B gives current in the muscle from B to A (= a current of injury).

After which moderate heat at B gives hardly any effect, because B is injured.

Dead (*i.e.* heat-rigored) muscle gives small effects in the same direction, that are ordinary physical (thermo-electric) effects.

The results on muscle are nearly quite satisfactory, the only drawback to their absolutely conclusive character is this small residual positive effect in the same direction as the ordinary thermo-electric currents from the heated side. But as will be understood from the consideration of skin effects, even this doubtful feature is eliminated when muscle-effects and skin-effects are studied comparatively.

	A	B
I. Muscle—		
Heat	←——	——→
Excitation or injury	━━→	←━━
II. Nerve—		
Heat	←——	——→
Excitation or injury	━━→	←━━
III. Skin—		
Heat	——→	←——
Excitation	←━━	━━→

The arrows under A and B indicate the direction of currents *in the tissue* in response to local warmth or local excitation at A and at B respectively: *e.g.*, if muscle, led off at A and B to the galvanometer, is heated at B, there is current in the galvanometer from B to A, in the muscle from A to B as indicated by the first arrow under B. Following the usual phraseology, we say that B becomes "positive." The second arrow under B indicates that there is current from B to A in the muscle when B is rendered active by injury or excitation, or, according to usual phraseology, B becomes "negative."

The arrows opposite the nerve indicate the analogous currents by local warmth or by local excitation, identical in direction with those of muscle.

The local skin currents both to heat and to excitation are of reverse direction to those of muscle (and of nerve), *e.g.*, if skin led off by electrodes A and B applied to its external surface is warmed at B, there is current in the galvanometer from A to B ("ingoing" current at B, or B "negative" to A). If it is excited at B, there is current in the galvanometer from B to A ("outgoing" current at B, or B "positive" to A).

In the foregoing description the ordinary terms "negative" and "positive" have been used. For my own part I find it conducive to clearness to think of the active spot B as "electro-positive," giving current from B to A in the tissue, rather than as "negative," giving current from B to A through the galvanometer, and I call B "zincative." But the least ambiguous description of direction of current between A and B is afforded by the arrows.

II. *Of Nerve.*

A nerve disposed in a similar manner gives similar results, with this difference, that the positive response to heat is relatively more evanescent and more easily replaced by the negative response to injury. This disappearance may occur so rapidly that a photograph taken after a few trial deflections may exhibit only a series of injury responses (negative with increasing negativity), the heat responses (positive with increasing negativity) having occurred with the trial deflections. It is advisable, therefore, to take nerve-photographs without any preliminary trials, as will be obvious on consideration of the record given herewith.

III. *Of Skin.*

Frog's skin, which, according to my previous observations,[*] invariably responds to local excitation by an outgoing current when led off by its external, but not by its internal, surface, is a particularly satisfactory tissue upon which to study the electrical effects of heat, for the electrical sign of the local effect of excitation is the reverse of that of muscle or nerve, and it possesses an effective (external) surface and an ineffective (internal) surface that can be separately tested.

Experiment.—Two unpolarisable electrodes A and B, in contact with the external or effective surface of the skin to a galvanometer. A stirrup of platinum wire in an accumulator and metronome circuit as for muscle.

Heat under B gives a large effect in the negative direction, indicating current in the skin from B to A (= B zincative), *i.e.* in the contrary direction to that of the current aroused by electrical or mechanical excitation.

Repeating the trial with the skin turned round so that the electrodes A B are in contact with its inner ineffective surface. Warmth applied as before to B gives little or no deflection; the deflection, if any, is in the opposite (negative) direction.

A killed piece of skin gives little or no deflection from the warmed spot B; the deflection, if any, is small and in the positive direction.

Thus in the living skin as in living muscle a current is aroused by warmth which is antidrome to the current aroused by electrical excitation; the facts in the two cases are as follows:—

	Local excitation of A.	Local warmth to A.
Muscle	A negative	A positive
Skin	A positive	A negative

Photographic records of the electrical effects of heat upon muscle, nerve, and skin. The connections in the three cases are with two points, A and B, as given above, heat being in each case applied at A or at B, as indicated by the signal marks *h*, *h*, giving in the case of muscle and in that of nerve heated at B response in the direction from A to B, and in that of the skin from B to A.

I. The muscle record consists of three successive positive responses to heat at A (A = antizn.); the general after-effect is in the negative direction, followed by four positive responses to heat at B (B = antizn.); the general

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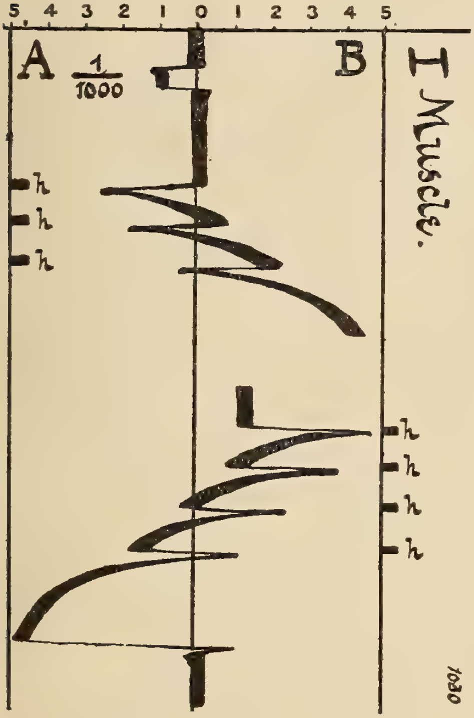
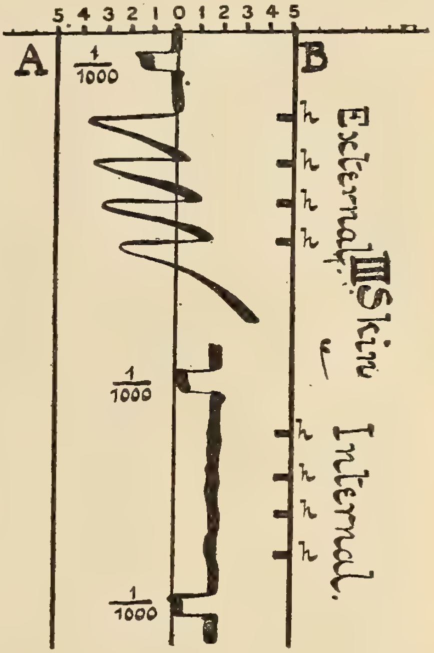
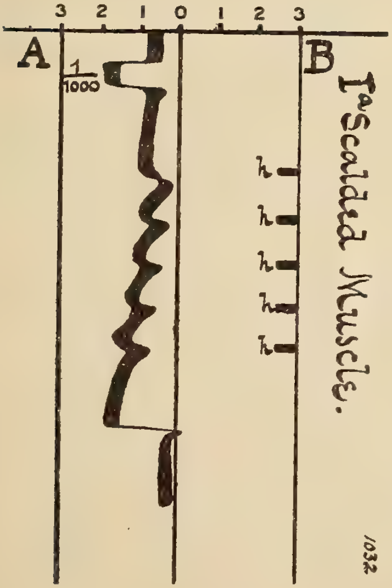
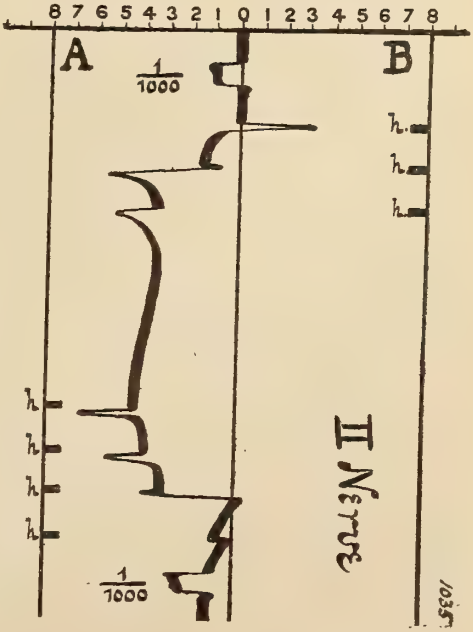
[*] Waller, 'Roy. Soc. Proc.,' vol. 68, p. 480, 1901, "Signs of Life."









after-effect is negative. The muscle is then scalded, and submitted to five successive glows as before; when a smaller deflection in the positive direction occurs at each glow. It is presumably a physical (thermo-electric) effect.

II. The nerve record consists of three successive effects of three successive glows under B, followed by four successive effects of four successive glows under A. In the B group the first response is positive, the second is small positive followed by large negative, and the third is negative. In the A group the first (positive) response is the largest, the second (positive) is smaller, the third is smallest and gives place to a deflection in the negative direction, due to injury, the fourth is a small negative deflection.

III. The skin record consists of two groups of four successive responses; in the first group the external surface, in the second group the internal surface, of the skin is led off to the galvanometer. In the first group, each response at B is relatively large and the current is directed in the skin from B to A ("ingoing" current, or B zincative). In the second group the responses are hardly perceptible.

In muscle (and in nerve), where the electrical effect of local excitation is "negative," the effect of moderate heat is "positive."

In the skin, where the electrical effect of local excitation is "positive," the effect of moderate heat is "negative."

Excessive heat, producing injury, gives a "negative" effect in muscle (and nerve), a "positive" effect in the skin.

N.B.—"Negative" = "Zincative." "Positive" = "Antizincative."

To my understanding, the expressions "positive" and "negative" are ambiguous without this specification, and the description given in the text in terms of "positive" and "negative," while correct, is very confusing.

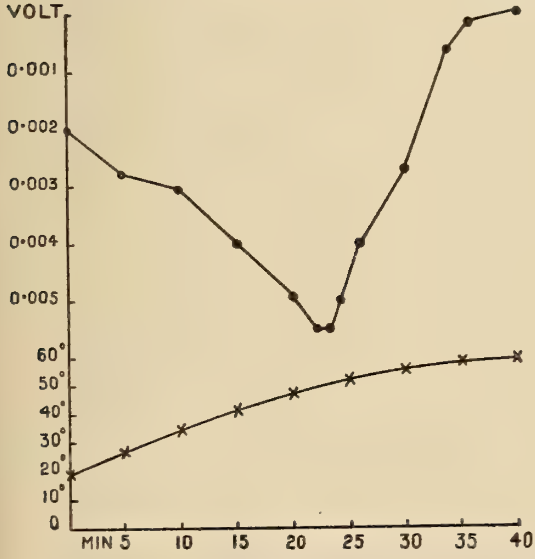
The general conclusion from all these experiments is that the first electrical effect of moderate local heat is of opposite direction to that of local excitation and of local excessive heat, *i.e.* that the effect of moderate heat is "*anti-excitatory.*"

[*Postscript* (*added May* 27).—In consequence of a question put to me at the conclusion of the demonstration that accompanied the above communication, I have taken observations on the effects of gradual rise and fall of temperature upon the normal (ingoing) current as well as upon the electrical response of the frog's skin. The alterations of temperature were brought about by gradually warming and cooling a metal box containing the skin and electrodes, the thermometer giving the temperature of the air in the box.

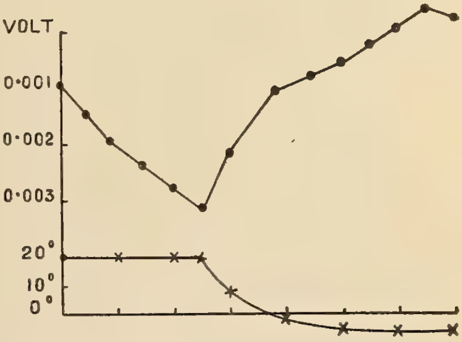
With rising temperature the electrical response of the skin increased at first and subsequently diminished, disappearing at a box temperature of between 45° and 50°. The normal (ingoing) current at first increased and subsequently diminished.

With falling temperature the electrical response diminished progressively, being altogether abolished at a box temperature of between −4° and −5°. The normal ingoing current diminished progressively with the fall of temperature. At the box temperature of 4° to 5° a sudden diminution of the ingoing current (*i.e.* an outgoing effect) was sometimes witnessed, which I attributed to an excitation occurring at the point of congelation. But in other cases mere irregular deflections were seen at this point.

Thus, as may be seen from the accompanying plotted curves, the effect of heat and of cold was in the same (outgoing) direction; the increased ingoing effect at the first application of heat being no greater than the spontaneous increase that takes place without alteration of temperature.]



Effect of gradual rise of temperature upon the normal skin current. Upper line = E.M.F. readings by compensator; lower line = temperature curve.



Effect of gradual fall of temperature upon the normal skin current. Upper line = E.M.F. readings by compensator; lower line = temperature curve.

The Incidence of Cancer in Mice of Known Age.

By E. F. Bashford, M.D., and J. A. Murray, M.D., B.Sc., Imperial Cancer Research Fund.

(Communicated by Prof. J. Rose Bradford, Sec. R.S. Received April 30,—Read May 20, 1909.)

The opportunity of obtaining accurate information of the frequency of spontaneous cancer in mice at different age-periods has presented itself in the course of a prolonged inquiry into the possibility of hereditary transmission of a liability to cancer. We have approached the question of heredity experimentally by breeding systematically from mice spontaneously affected with malignant new growths, and propose to determine the frequency of spontaneous cancer in mice in whose ancestry the disease has occurred with varying frequency. This investigation is still in progress and cannot be reviewed profitably for several years, but the data which have so far accumulated are of sufficient interest, in their bearing upon the statistical and biological importance of the age-incidence of the disease, to warrant a preliminary account being published; although the small numbers at present available still render the greatest caution necessary.

The method by which the data have been obtained is as follows: Mice spontaneously affected with cancer are not killed when brought into the laboratory, but the tumours are excised and used for transplantation. The clinical course, the microscopical examination, and the results of transplantation of the tumours, together with the *post-mortem* examination of the animals, give the best security for the correctness of the diagnosis of cancer. It is only under these precautions that the breeding experiments have proceeded. Each spontaneously affected mouse, or pair mated for breeding, has been housed in a separate cage. The cages have been sterilised and changed at regular intervals. In the first instance, the males mated with these spontaneously affected females were the offspring of spontaneously affected animals received pregnant. Later, males bred in the laboratory from cancerous parents were used, so that the pedigrees constructed for the later litters show some strains with a relatively enormous preponderance of cancerous ancestors. When a litter is born each young mouse receives a number, the date of birth is entered in a list, and the sex and colour or other distinguishing marks noted against each. So soon as they are able to look after themselves the litters are separated from the mother, and the males and females segregated in fresh cages. It is thus possible to distinguish each animal born in the laboratory by reference to an index, which at once gives the ancestry, the date of birth, and the age of the animal in question. The mice have been systematically examined daily.

There is difficulty in obtaining offspring from mice suffering naturally from cancer, and the breeding experiments began to be regularly successful in January, 1907. The first case of cancer was found in a female mouse in March, 1908, the animal being nine months old. Since that date 18 additional spontaneous malignant new growths have been obtained. Every case has been subjected to careful microscopical examination, and only undoubted cases of malignant new growth are reckoned. So far all have occurred in female mice, and with two exceptions have affected the mamma. The exceptional cases were one of generalised malignant lymphoma, and one of melanoma or melanotic sarcoma of the external ear. The remainder were carcinomata of the mamma in which the adenomatous character was present in varying degree; in two of them small areas of keratinisation were found in the sections examined.

It was of interest to determine at what age the tumours were *first observed*, and to determine the number of animals of the same age under observation. As no cases of new growth have yet occurred in the males* bred, these are excluded from the present purview. The females were distributed by means of a card index into five groups differing in age from each other by intervals of three months. Animals under six months old have been excluded because of the high mortality in the first six months of life from infectious diseases of all kinds (pneumonia, enteritis, septicæmia), and because the youngest mouse in which a true malignant new growth occurred was exactly six months old. It will be noted from the table given below that no female animal attained the age of two years. On April 26, 1909, a census of the females was taken, and all those which had died over six months old, since the beginning of the experiment, were added to the corresponding age-groups. The mice which were then still living, after developing cancer, and those which had died from the disease, appear in the age-group corresponding to the age at which the disease was first discovered in them. The percentages of the following table are therefore not strictly comparable with death-rates, but are to be read as giving the liability to cancer at different age-periods.

The progressive increase shown in the table presents a remarkable correspondence with the facts long familiar to students of the incidence of cancer in the human subject. It furnishes a striking confirmation in the mouse, of the conclusion we advanced in 'Roy. Soc. Proc.,' vol. 73, January, 1904, and in the First and Second Scientific Reports of the Imperial Cancer Research Fund, that, in animals as in man, the *recorded* frequency of cancer varies with the opportunities for examining a large number of adult and aged individuals.

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* Of the first 1145 mice bred, 588 were males and 557 females. The preponderance of cancer in the female is due to the great liability of the female to cancer of the mamma.

Age.	6—9 months.	—12 months.	—15 months.	—18 months.	—21 months.	—24 months and over.
Total	135	110	94	21	6	—
Cancer	3	4	7	3	2	—
Per cent.	2·2	3·5	7·4	14·2	33·3	—

Account was taken of the age-incidence of cancer in the human subject in the hypotheses of Thiersch* and of Cohnheim,† which were formulated for man only, and are untenable to-day. The general biological significance of the age-incidence of cancer, for which we have so often argued, has been ignored, or, when mentioned, minimised by most pathologists, and, in recent years, also by those engaged in the experimental study of the disease. It is, perhaps, not too much to hope that the foregoing presentation of the facts will henceforward impress on those engaged in the investigation of cancer the urgent necessity for precise knowledge of the ages of men or animals in whom the incidence of cancer is being studied. In particular, the difference between mice 15 months old and 21 months old in their liability to cancer at once invalidates completely all statements of the relatively greater frequency of cancer in one group of mice than another, when the exact age of the animals is not known. The same objection must be raised to assertions of the occurrence of epidemics in other animals. Such statements have been frequently made, and have received wide currency since experiment demonstrated the possibility of the artificial transmission of cancer from one animal to another of the same species, but only, however, by implanting living cancer-cells, and also demonstrated that this form of transmission could not be made responsible for the great frequency of malignant disease. The above criticism therefore applies with destructive force to all statements which have appeared up to the present on the occurrence of epidemics of cancer in mice and rats. Until it can be shown that the conditions of experiment have altered the normal age-incidence of the disease, the theses which have found such ready acceptance must be regarded as not proven.

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* Thiersch, 'Der Epithelialkrebs, namentlich der Haut,' Leipzig, 1865.

† Cohnheim, 'Vorlesungen über allgemeine Pathologie,' Berlin, 1877, 2nd Edition, 1882.

As in the case of other communities of mice in outside breeding establishments, our stock, at present under consideration, is a highly in-bred one. It is not profitable at present, considering the small number of tumours which have been obtained, to analyse the cancerous and non-cancerous individuals with reference to this factor or to the ancestry.

The positive value of these observations lies in the statistical confirmation they bring to the results of the comparative histological and biological studies of the Imperial Cancer Research Fund, which have shown the close parallel, amounting in many particulars to complete identity between malignant new growths in man and other vertebrates. They demonstrate that the law of the age-incidence of cancer holds also for the shortest-lived mammals as it holds for man. Since the facts accord with the imperfect data we have elicited for other vertebrates, they make the general applicability of the law of age-incidence probable, and therefore any explanation of the etiology of cancer must accord with the circumstance that, when considered *statistically*, cancer is a function of age, and when considered *biologically* is a function of senescence.

The Electrical Reactions of certain Bacteria, and an Application in the Detection of Tubercle Bacilli in Urine by means of an Electric Current.

By Charles Russ, M.B.

(Communicated by A. D. Waller, M.D., F.R.S. Received May 21,—Read June 24, 1909.)

Introduction.

The aim of the following experiments was to ascertain whether bacteria suspended in an electrolyte through which a current passes are transmitted to either electrode, and if so, whether pathogenic organisms could be collected and extracted by such means from pathological liquids.

Method of Experiment.

The first observations as to a possible migration of bacteria under the action of an electric current were made in the following way :—

A cover square was fitted with two platinum foil terminals, separated about 6 mm. from each other. A drop of weak bacterial emulsion made electrical connection between these two terminals, and was prevented from evaporating by another cover square resting on the top of the first one, the edges of which were greased; the "glass cell," as it may be called, was then mounted on a stand (fig. 1), which rested on the stage of a microscope, and a current of about 1 milliampere sent through it.

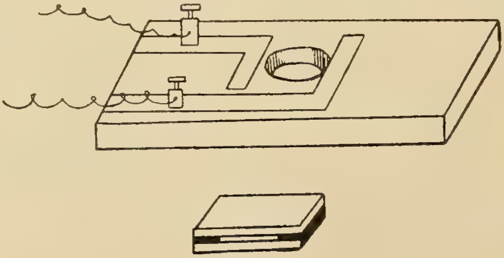


Fig. 1.

It was at once found that the bacteria, viz., *Staphylococcus aureus*, *Streptococci*, *M. melitensis*, *B. tuberculosis*, *B. coli*, *B. typhosus*, *B. of Gaertner*, *B. pyocyaneus*, and *Hoffmann's bacillus*, moved towards one electrode, their direction of movement being reversed on reversal of the current; the velocity of transmission was approximately estimated at about 1/100 mm. per minute (under the particular conditions of pressure and sectional area).

It was a matter of considerable difficulty to keep a particular bacillus continuously in view and thus watch its migration; to obviate this, and in view of the possibility of the observed movement being due to some variation of surface tension of the fluid under the influence of the current, the experimental arrangements were altered and observations made in the following way:—

An emulsion of the *Bacillus coli* was made by pouring normal saline upon a fresh agar culture of the organism, sweeping the growth by a platinum loop into the saline, and pouring it into a test-tube, which was then sealed in the blow-pipe; by thorough agitation an even emulsion was made; some of this emulsion was then poured into a glass U-tube, fitted with platinum terminals which just dipped beneath the liquid; a current from Leclanché cells was sent through the emulsion. Observations at frequent intervals showed that the bacilli were accumulating under the anode in one limb of the U-tube; after several hours a dense column had formed, the fluid in the opposite limb becoming clear; reversing the current reversed the direction of motion of the bacteria.

A systematic series of tests was next undertaken with different bacteria in various electrolytes. To expedite the work, four similar U-tubes were mounted on a stand as seen in fig. 2, and observations made simultaneously of the behaviour of the bacteria when suspended in different electrolytes, the same current traversing the four solutions in series. The current was measured by a tangent galvanometer in circuit, and the voltage between the terminals of the U-tubes by a voltmeter when required. Usually 4-per-cent. solutions of the electrolytes were used; with weaker solutions, *e.g.*, ½ per cent., gravitation of the bacteria to the bottom of the U-tubes occurred before the electrical effect was evident.

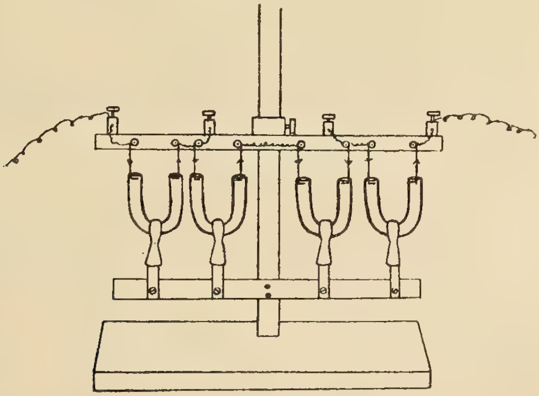


FIG. 2.

It was thus found that:—

(1) The accumulation at an electrode varies in degree with different species in the same electrolyte.

(2) The accumulation at an electrode varies for the same species in different electrolytes.

Table I.

	− Na_2.	+ SO_4.	− Na.	+ NO_3.	− Na_2.	+ HPO_4.	− Na.	+ Cl.
B. coli communis		*		*		*		*
B. typhosus		*		*		*		*
Staphylococcus aureus		Feeble *		Feeble *	Not tested			*
Tubercle bacillus	Nil		Nil		Nil			Feeble *
Hoffmann's bacillus	,,		,,			*		*
Pyocyaneus, B.		*		*		*		*
M. melitensis		*		*		* *		*
Streptococcus	Nil		Not tested			*		*
Gaertner's bacillus		*		*		*		*

The acid radicle column = anode.
base ,, = cathode.
* indicates aggregation.
** = marked aggregation.

Table I contains the results of a number of observations made with different bacteria in four electrolytes which all contained the same basic element (sodium), but different acidic elements.

A similar set of observations was carried out with four electrolytes having the same acid radicle (SO_4), but different basic elements, and the results collected in Table II.

Table II.

	− Zn.	+ SO_4.	− K_2.	+ SO_4.	− Mg.	+ SO_4.	− $(NH_4)_2$.	+ SO_4.
B. coli communis	Nil			*	Nil		Nil	
B. typhosus	,,			*	,,		,,	
Staphylococcus aureus	,,			*	? *		Feeble *	
Tubercle bacillus	,,		Nil		? *		*	
B. pyocyaneus	,,			*	Nil		Nil	
B. Gaertner	,,			*	,,			Feeble *
M. melitensis	Feeble *			*	,,		*	

The streptococcus in Table I died before Table II was begun.
The ? was necessitated by oxide formations referred to under choice of electrolytes.

It was important to decide the relationship of these bacterial movements to the bacterial vitality. In point of fact the accumulation occurs in boiled, *i.e.* dead, as well as in living cultures. As regards the probable nature of the movement of the bacteria under the action of the current, we are in the presence of the following alternatives:—

(1) If bacteria possess electric charges, they might be attracted to the electrode of opposite sign, and migration of the bacilli would ensue.

(2) Minute particles suspended in fluids, through which a current is sent, are known to be directed to the electric terminals. During the course of this work solutions have been found in which no movement of the bacilli was effected by the current. It will be seen from some experiments to be described, that the phenomena are hardly to be explained on the lines indicated above.

(3) When a current passes through an electrolyte, the usually accepted view of the main processes therein involved is that there is a movement through the solution of the ions of the solute in opposite directions towards the electrodes. If we suppose that some chemical affinity exists between the ions and the bacteria, there is at once the possibility of migration of the bacteria towards the electrode; the transmission of the bacteria to the anode in one electrolyte, and the cathode in another (*e.g.*, the tubercle bacillus proceeds to the anode in sodium chloride, and to the cathode in ammonium sulphate) can be attributed to a chemical affinity between them and the ions of the electrolyte.

The migration of bacteria in electrolytes having been established, the next step was to ascertain whether bacteria present in very small numbers in a pathological liquid would be transmitted to either electrode in an electrolyte, and thus concentrated in a small volume of liquid for further examination.

With this object in view, the tubercle bacillus in urine was chosen because of the convenience in recognising the organisms without there being any necessity of undertaking cultivation work. The chief conditions required for the purposes of these experiments are that the electrolyte (1) should conduct electricity well, (2) should not be destructive of organic matter, (3) should form a colourless fluid in aqueous solution, (4) should yield no metal or metallic oxide as a result of electrolysis; for such action has been found to mask bacterial aggregation.

The search for a suitable electrolyte for the tubercle bacillus proved a long and laborious one. The general method of experimenting was practically identical with that described in which the U-tubes were used. The tubercle bacilli from glycerine agar cultures were ground in a mortar, made into an emulsion with water and added to the electrolytes under test. Out of

43 substances (a list of which is appended) tried in this way, a decided aggregation was observed with ethylamine, ammonium sulphate, acetamide, and iodide of potash. The first three will be noticed to contain an NH_2 or NH_4 group. The aggregation in acetamide or potassium iodide is anodic, but weaker than the cathodic aggregation in ethylamine, or ammonium sulphate.

A curious effect was noticed with ammonium sulphate, viz., an early aggregation at the cathode, soon followed by a sinking away of the massed organisms, resulting in the appearance of a clear zone between the bacterial cloud and the platinum foil. This was probably the result of some secondary electrolysis at the terminal in question; this disturbing action led to the abandonment of ammonium sulphate for the purposes of these experiments.

A good accumulation was found to occur with a mixture of ethylamine and lactic acid, the migration of the bacilli being towards the cathode; separate tests indicated no reaction in lactic acid, and a moderate one with ethylamine alone. The movement of the tubercle bacillus in normal urine was found to be slightly anodic. The addition of urine to the lactic acid and ethylamine did not interfere with the aggregation of the tubercle bacilli at the cathode. At the end of the test the presence of the bacilli in large numbers at the cathode, and their absence at the anode, was confirmed by making stained films from the cloudy and clear fluids round the respective poles.

It was, however, noticed in these films that the bacilli stained feebly after several hours of electrolysis in the ethylamine lactic acid and urine mixture; this difficulty was overcome by the addition of bromic acid to the mixture.

The proportions of these substances found to be most satisfactory were:—

Ethylamine, 5-per-cent. solution………	One part.
Lactic acid, 10 " ………	Four parts.
Bromic acid, 5 " ………	Two parts.
Urine ……………………………………	One or two parts.

It may be useful to mention here that when electrolytes are added to urine, and the mixture electrolysed, there is formed at the cathode a white flocculent substance which is insoluble in the fluid, but soluble in mineral and organic acids. As this substance is easily mistaken (in aggregation tests in urine) for the bacterial cloud expected at the electrode, the solubility in acid should be tested for. It may further be distinguished by its rapidity of appearance and bulkiness of the cloud, in which particulars it contrasts with the true bacterial massing at the foil; the lactic acid used in the mixture above mentioned prevents its formation.

In order to apply the observed aggregations of tubercle bacillus as a method of their extraction from tuberculous urine, the experimental arrangements were altered as follows:—

The modified U-tube (fig. 3) was filled with a mixture of tuberculous urine, ethylamine, lactic and bromic acids, thoroughly shaken previously. In the narrow limb of the vessel a platinum foil strip was submerged, and served for the transmission of current through the main column of fluid. The gases liberated at this foil escaped by the narrow limb, and were thus prevented from traversing the bulk of the fluid. In the broad limb a glass tube was submerged slightly and traversed by a platinum wire, which just touched the fluid surface. The lower end of the glass tube formed a bacterial trap by arranging the circuit so that the platinum wire was the cathode. On passing a current the tubercle bacilli contained in the tuberculous urine in the vessel are conveyed to the cathode as in the U-tube experiments; they eventually enter the fluid enveloped by the submerged end of the tube, and remain close to the platinum wire. After sufficient time the wire was carefully lifted out, the glass tube slightly lowered in the vessel, the top of the tube closed by the wet or greased finger, and lifted away to a glass slide, and examined for tubercle bacilli. The fluid contained in the trap was 2 or 3 minims in volume, and strongly alkaline at the end of the process, the bulk of the mixture remaining acid. This alkaline fluid was acidified with a 10-per-cent. solution of acetic acid on the slide previously smeared with albumen fixative; it was fixed by heat and stained by the ordinary Z N carbol fuchsin method. Tubercle bacilli were found in small numbers in the early attempts.

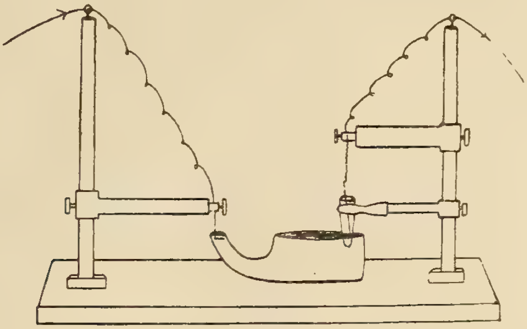


FIG. 3.

The following experiment, in which the centrifuge and the electric current were compared in the detection of tubercle bacilli placed in very small numbers in a test fluid, is given in illustration of the power of the electrical method:—

My colleague, Dr. Fletcher, prepared an emulsion of tubercle bacilli in water, and standardised to contain 1000 bacilli per cubic centimetre; he thoroughly mixed ½ c.c. (*i.e.* 500 "T. B.") of this emulsion with 100 c.c. of normal urine. Allowing this mixture to stand for 24 hours in a conical vessel to assist sedimentation of the bacilli, he then siphoned off the supernatant three-quarters of its volume; the remainder was centrifugalised (1/8 H.P. electric centrifuge) three times. In a stained preparation from the final deposit he found *no tubercle bacilli.* I added ½ c.c. (*i.e.* 500 tubercle bacilli) of the same emulsion to 50 c.c. of normal urine, and made up the volume to 100 c.c. by adding bromic acid, lactic acid, and ethylamine, and thoroughly shaking the mixture. This mixture was electrolysed in the vessel illustrated in fig. 3. After 21 hours' electrolysis 128 tubercle bacilli were counted in the stained preparations made from the trap contents.

Detection of "T. B." in Tuberculous Urines by the Centrifuge and Electric Current.

Case.	Centrifuge.		Electric current.		
	Volume of urine used.	Description of "T. B." seen in films.	Volume used.	No. of times diluted.	No. of "T. B." seen in film but which is not a total count.
	c.c.		c.c.		bacilli.
1	10	Moderate numbers ...	10	15	59
2	30	,, ...	10	5	38
3	repeat	...	7	6	205
4	20	Fairly numerous	6	7	84
5	20	...	6	7	149
6	20	...	6	7	103
7	20	...	2	21	15
8	10	Fairly numerous	5	9	22
9	10	...	5	9	68
10	10	...	5	9	35
11	10	...	5	9	40
12	10	...	10	10	65

The volume used by centrifuge = 10 c.c. once, twice or thrice repeated; *i.e.* the capacity of the tube = 10 c.c.

The film forms a permanent record of the experiment in each case.

These experiments concluded by repetition of this process in 12 cases, using always tuberculous urine, in which the bacilli had been previously found by the centrifuge. During these cases, variations of the time the current passed and of its strength were introduced. The results are collected in Table III, the main conclusions being:—

(1) Tubercle bacilli were obtained in the trap upon the conclusion of every test.

(2) They were present in larger numbers than would have been obtained in 2 or 3 minims of the original urine.

(3) The transmission of the bacilli by the current is emphasised by the relatively large number of bacilli obtained in the trap when working with very much diluted solutions of the original urine.

(4) The evidence is not complete that all the bacilli present in the urine used were conveyed into the trap (though examinations of the "catch" were made at successive stages of the electrolysis).

The appearance of the bacilli in the films made from the trap is similar to that in a film from centrifuged urine, though the pus cells undergo dissolution with increasing alkalinity of the trap contents. If the electrolysis was carried on for 24 to 36 hours the bacilli failed to take the stain properly; such exposure, however, is unnecessary.

I am indebted to Mr. S. Russ, Demonstrator of Physics, Manchester University, for assistance with the electrical technique and measurements; to Dr. Eastes for the use of his laboratory during the earlier part of the experimental work, and to Mr. Pardoe for tuberculous urine from St. Peter's Hospital.

In conclusion, the results of the present preliminary investigation may be summarised as follows:—

Certain bacteria under the influence of a suitable current aggregate at one or other electrode. The aggregation varies with the nature of the electrolyte, and is probably due to affinity between the products of electrolysis and the bacteria. It occurs with killed as well as with living bacteria. The aggregation by electrical currents affords a means of collection and examination. The differences in behaviour of various bacteria are such as to suggest the possibility of utilising the method for purposes of specific discrimination; but in this particular the data hitherto obtained are not sufficient to warrant definite statements.

List of Electrolytes in which Reaction of Tubercle Bacilli was sought.

Inorganic.—Potassium iodide, iodic acid, sodium sulphite, sodium carbonate, sodium bicarbonate, sodium nitrite, ammonium magnesium phosphate, potassium chlorate, microcosmic salt, ammonium magnesium sulphate, potassium cyanide, and copper sulphate.

Organic.—Sodium tartrate, sodium acetate, sodium citrate, maltose, glucose, lactose, cane sugar, ethyl alcohol, methyl alcohol, acet-aldehyde, glycerin, formalin, formic acid, amido-acetic acid, urea, uric acid, sodium urate, oxalic acid, urea nitrate, lactic acid, acetone, chloral hydrate, phenylene-di-amine, sulphanilic acid, picric acid, carbol fuchsin, chloroform, carbolic acid, acetic acid, and acetamide.

[*Note.*—Since completing these experiments I have searched the literature of the subject with the following results :—

Abbot and Life (' American Journ. Physiol.,' 1908, p. 202) tested microscopic quantities of motile bacteria suspended in distilled water, and observed to-and-fro movements in a glass trough. They used excessively minute currents, and concluded that variations in the observed reactions of the organisms depended upon their cultivation in acid or alkaline media. They found no effects with dead or non-motile (living) species; they avoided currents large enough to cause electrolysis, and concluded that their results were galvanotropic.

Apostoli and Laquerriere (' Compt. Rend.,' 1901, vol. 133, p. 186) concluded that constant currents are able to sterilise cultures or attenuate their virulence. They studied especially the conditions necessary to produce such effects.

S. Krüger, "Ueber den Einfluss des constanten electrischen Stromes auf Wachsthum und Virulenz der Bacterien" (' Zeitschrift für Klinische Medicin,' 1893), contributes an account of the lethal effects of electricity applied to bacteria.

Other references are appended to papers I have not yet been able to consult, viz. :—

Friedenthal, H. "Ueber den Einfluss des elektrischen Stromes auf Bakterien," ' Centralblatt f. Bakteriol.,' 1. Abt., Jena, 1896 (vol. 19, pp. 319—324); "Ueber den Einfluss der Inductions-Electricität auf Bacterien," *ibid.* (vol. 20, p. 505).

Jennings, H. S. Papers on reactions to electricity in unicellular organisms. ' J. Comp. Neurol. and Psychol.,' Granville, vol. 15, 1905, 528—534.

Bang, S. "Wirkungen des elektrischen Bogenlichtes auf Tuberkelbazillen in Rein-Kultur," ' Mitt. Finsens Lysinst. Kopenhagen,' Leipzig, H. 3, 1903 (97—112).]

Trypanosoma ingens, n. sp.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service.

(Received April 30,—Read May 20, 1909.)

(Sleeping Sickness Commission of the Royal Society, 1908—09.)

[PLATE 7.]

This is such an extraordinary looking parasite that the Commission thinks it deserves a short preliminary note, a name, and to be figured.

The name is taken from Virgil's description of the Cyclops, *informe, ingens*. It was first discovered in the blood of a reed-buck on February 13, 1909, at Namukekera, Uganda (lat. 0° 40′ N.; long. 32° 15′ E.), the estate of the Uganda Company, Limited; then in a bush-buck, and lastly in an ox. The wild animals and the cattle feed in the same pastures, so that it is not remarkable that the oxen should become infected.

At present it is not known what the carrier is, and this will probably be a difficult thing to determine. Collections of the blood-sucking flies and ticks are being made on the Namukekera Estate, and this may lead in time to the discovery of the carrier. Up to the present the following list includes all the blood-suckers found in this particular district:—

Chrysops distinctipennis, Austen.
Stomoxys calcitrans, Linn.
Stomoxys nigra, Macq.
Tabanus tæniola, Pal. de Beauv.
Hæmatopota unicolor, Ricardo.
Hæmatopota, sp. nov.
Hæmatopota brunnescens, Ricardo.

Trypanosoma ingens, when seen alive in a fresh preparation, moves slowly and deliberately across the field of the microscope, with a fine rippling, or at times a broader undulating movement.

In stained preparations this huge trypanosome may measure as much as 122 microns, and even then it is lying in such a formless huddled-up way among the red blood corpuscles that it looks capable of stretching out to a much greater length. The other specimens figured measure 72, 77, 88, and 82 microns. The breadth is 7 to 10 microns.

The micronucleus is small and round. It measures about a micron in diameter. It lies posterior to, and quite close to, the nucleus. From it, in well-stained specimens, a well-marked, though narrow, undulating membrane arises, which runs to the anterior extremity and ends in a free flagellum.

The nucleus is oval in form, and lies across the body. It is situated nearer the posterior end than the anterior, and in our specimens has stained a pale pink.

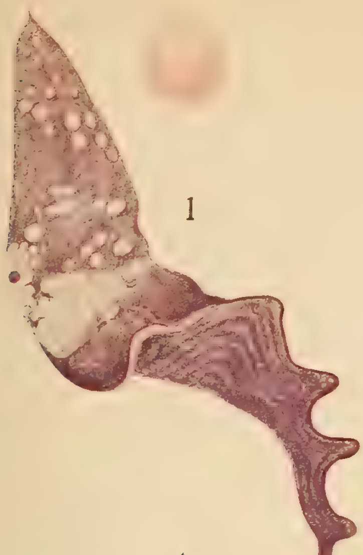
The body substance is markedly granular behind the nucleus, while in front the structure described as myonemes is particularly well marked.

More minute measurements of one of these trypanosomes are as follows:—

	microns.
Posterior end to micronucleus	18
From micronucleus to nucleus	4
Nucleus: long diameter, 8 microns; short diameter	4
Nucleus to anterior end	40
Free flagellum	17
Total	83

It is unnecessary in this preliminary note to go more fully into the structure of this trypanosome, or to describe it at greater length. An examination of the coloured drawings reproduced in Plate 7 will give a more distinct idea of its appearance than any written description.

The drawings were made by Lady Bruce, R.R.C. Figs. 1, 3, and 4 are from reed-buck, fig. 2 from the ox. All are magnified 2000 and stained Giemsa.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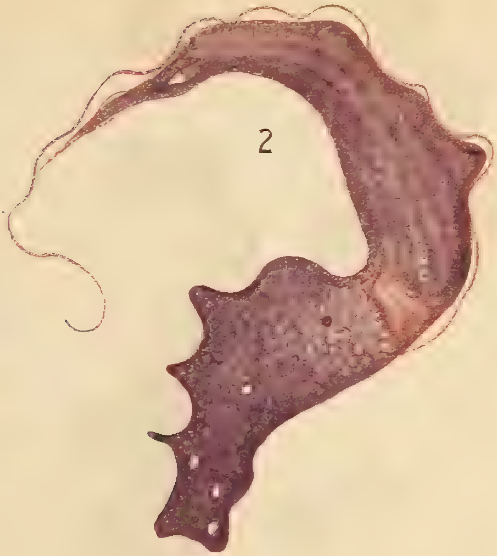
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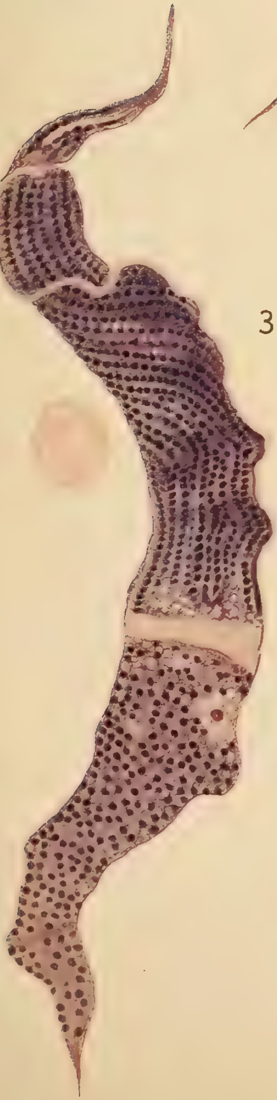
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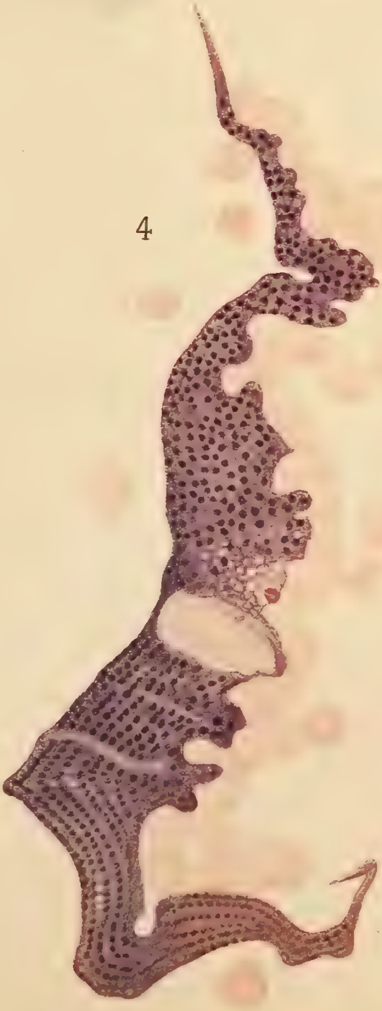
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The Effect of the Injection of Intracellular Constituents of Bacteria (Bacterial Endotoxins) on the Opsonising Action of the Serum of Healthy Rabbits.

By R. TANNER HEWLETT, M.D.

(Communicated by Prof. W. D. Halliburton, F.R.S. Received June 5,—Read June 24, 1909.)

In a series of researches the late Dr. Allan Macfadyen studied the properties of the intracellular constituents of bacteria and other organisms obtained by mechanical trituration of the organisms in the presence of liquid air. He showed that the cell juices thus obtained are:—

(1) Toxic on injection into animals (*e.g.*, *B. typhosus*,* *Spirillum choleræ*,† *B. suisepticus*,‡ pneumococcus,§ and others).

(2) Capable of inducing the formation of anti-endotoxins on injection into animals (*e.g.*, *B. typhosus*,|| *Spirillum choleræ*¶) which possess protective and curative properties *in vivo*, and bacteriolytic properties *in vitro*.

(3) Cause the development of agglutinins (*e.g.*, *B. typhosus*** and yeast††). It was thought that it might be of interest to investigate whether the intracellular bacterial constituents are capable of inducing alteration in the opsonising action of the serum of normal rabbits.

The organisms selected were the *B. typhosus*, the *B. tuberculosis*, and the *M. pyogenes*, var. *aureus* (*Staphylococcus pyogenes aureus*). The intracellular constituents of these organisms were obtained by the Macfadyen method,‡‡ viz., by growing the organism on surface agar in Roux bottles, scraping off the growth, suspending this in sterile water, centrifugalising and collecting the bacterial paste on the walls of the centrifuge. The bacterial paste is weighed so as to ascertain the amount, and then ground in the machine after freezing.

After grinding, the ground material is made up with distilled water or with 0·1-per-cent. sodium hydrate, so as to form a 10-per-cent. solution

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* 'Roy. Soc. Proc.,' vol. 71, 1903, p. 77 (with Sydney Rowland).

† 'Lancet,' 1906, vol. 2, p. 494.

‡ 'Centralbl. f. Bakt.,' Abt. I (Originale), vol. 43, 1907, p. 143.

§ 'Brit. Med. Journ., 1906, vol. 2, p. 776.

|| 'Roy. Soc. Proc.,' vol. 71, 1903, p. 351, and vol. 77, 1906, p. 548.

¶ 'Lancet,' 1906, vol. 2, p. 494.

** 'Lancet,' 1906, vol. 1, p. 373.

†† 'Centralbl. für Bakteriol.,' Abt. I, vol. 30, 1901, p. 368.

‡‡ See 'The Cell as the Unit of Life' (Churchill, 1908), p. 274.

(calculated on the original weight of the moist bacterial paste), and filtered through a sterile Berkefeld filter. One to three cubic centimetres of the filtered solution are then dried *in vacuo* over sulphuric acid and weighed, so as to ascertain the weight of material contained in the endotoxin solution. This weight is regarded as the weight of endotoxin; actually the endotoxin is slightly less than is represented by this weight, in consequence of the presence of traces of salts. The amount of endotoxin having been thus ascertained, sufficient sterile 0·8-per-cent. sodium chloride solution is added to the filtered solution of endotoxin so as to form a 1-per-mille solution. All the operations are performed aseptically, in order to obtain a sterile preparation.

The rabbits were all large healthy animals, and blood was obtained in Wright's capsules from an ear vein. In all instances, the blood used as the control was taken at the same time as the samples from the inoculated animals, and the specimens for counting the number of bacteria ingested by the polymorphonuclear leucocytes were made in the usual manner within two to three hours after bleeding the animals. The leucocytes employed were *human* leucocytes, as rabbit's leucocytes were found to be less satisfactory for making the stained films, and the counts were made on 50 cells. All the inoculations of endotoxin, tuberculin, and vaccine were made subcutaneously in the back.

RESULTS OBTAINED.

A. *Typhoid endotoxin.*—The determination of the effect of injections of typhoid endotoxin on the opsonising action of rabbit's serum is complicated by the fact that agglutinins and bacteriolytic substances are formed which cause agglutination and solution of the organisms (typhoid bacilli) in the mixtures of serum, leucocytes, and organisms employed for preparing the films with which the counts are made. The results, therefore, in this case must be regarded as approximate only. The endotoxin was prepared from an avirulent strain of the typhoid bacillus. Three rabbits were taken, one being kept as a control, the two others each receiving a dose of 0·1 milligramme of endotoxin.

In addition to determining the opsonising action of the undiluted serum, the effect of dilution was also studied, for Klien*s has shown that dilution up to a certain point *increases* the opsonising action of human typhoid serum.

The following results were obtained:—

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* 'Bull. of the Johns Hopkins Hospital,' vol. 18, Nos. 195 and 196, 1907, p. 245.

Table I.—Number of Typhoid Bacilli ingested by 50 Polymorphonuclear Leucocytes.

Period.	Dilution of serum.	Control (opsonic index = 1·0).	Rabbit I.	Opsonic index.	Rabbit II.	Opsonic index.
Control before inoculation	undiluted	96	82	0·85	88	0·9
	1 in 5	60	54	0·9	54	0·9
	1 10	38	42	1·1	36	1·0
24 hours after inoculation	undiluted	112	20	0·17	28	0·25
	1 in 5	86	12	0·13	14	0·16
	1 10	54	0	—	0	
48 hours after inoculation	undiluted	122	166	1·4	156	1·3
	1 in 5	74	398	5·4	336	4·5
	1 10	43	154	3·6	166	3·8
3 days after inoculation	undiluted	112	357	3·2	369	3·3
	1 in 5	70	204	3·0	254	3·6
	1 10	35	114	3·3	116	3·3
	1 20	—	70	—	84	
5 days after inoculation	undiluted	196	392	2·0	327	1·7
	1 in 5	48	548	11·4	496	10·3
	1 10	33	515	15·6	448	13·6
	1 20	14	325	23·0	357	25·5
	1 50	—	101	—	48	
6 days after inoculation	undiluted	135	103	0·8	131	1·0
	1 in 5	64	270	4·2	160	2·5
	1 10	33	124	3·7	135	4·0
	1 20	—	98	—	75	
	1 50	—	83	—	80	
	1 100	—	66	—	29	
7 days after inoculation	undiluted	114	155	1·4	125	1·1
	1 in 5	49	110	2·3	71	1·4
	1 10	28	71	2·4	42	1·5
	1 20	—	50	—	60	
	1 50	—	35	—	29	
	1 100	—	29	—	22	
8 days after inoculation	undiluted	121	202	1·7	121	1·0
	1 in 5	110	240	2·2	145	1·3
	1 10	51	168	3·3	198	3·9
	1 20	—	164	—	126	
	1 50	—	98	—	45	
12 days after inoculation	undiluted	118	—		112	0·95
	1 in 5	60	—		112	1·9
	1 10	23	—		180	7·8
	1 20	—	—		164	
	1 50	—	—		98	
	1 100	—	—		28	

It is not suggested either in Table I or in Table II that the index is correct to the second decimal. The figure in the second decimal place is given only to indicate the *trend* of the index.

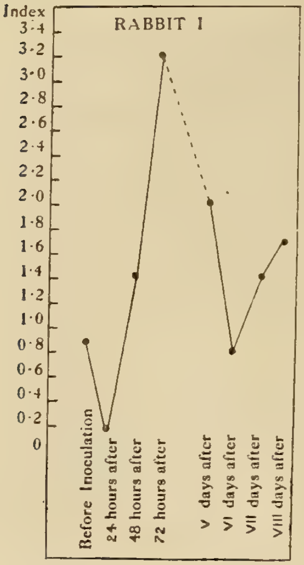

Chart I.—Opsonic Index after Inoculation with 0·1 mgrm. Typhoid Endotoxin.

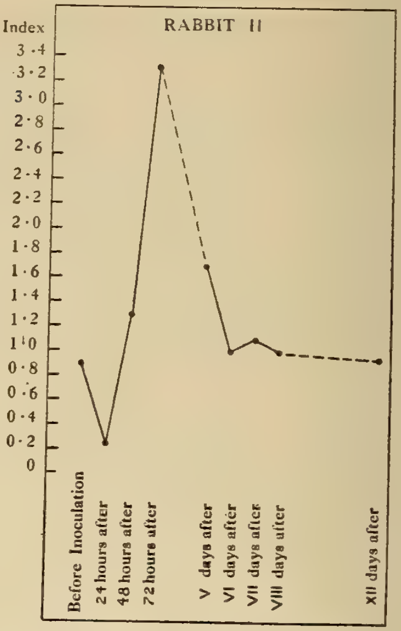

Chart II.—Opsonic Index after Inoculation with 0·1 mgrm. Typhoid Endotoxin.

From the foregoing table it is evident that an injection of 0·1 milligramme of typhoid endotoxin produces, 24 hours after inoculation, a considerable decrease in the opsonising action of the serum, that is a marked "negative phase" (Wright), followed by a considerable rise in the opsonising action of the serum which persists for some days. The opsonic index yielded by the undiluted serum is given in graphic form in Charts I and II. The dilution of the normal serum produces a decrease in its opsonising action, whereas a dilution of the serum of the inoculated rabbits produces an apparent increase in the opsonising action.

B. *Staphylococcus endotoxin.*—Three sets of experiments were carried out with the endotoxin of the *M. pyogenes*, var. *aureus* (*Staphylococcus pyogenes aureus*), viz., a comparison of the effects of the endotoxin derived from (*a*) an ordinary old laboratory strain of the organism, (*b*) a recently isolated strain, and (*c*) the effect of a vaccine prepared from the strain used for *a*, on the opsonising action of the serum of normal rabbits. Equivalent quantities (0·1 milligramme solid matter) both of vaccine and of endotoxin were given, and the endotoxin solution was prepared with 0·1-per-cent. sodium hydrate solution. The opsonising action of each serum, some time after inoculation, was also tested with both strains of organisms. The results obtained are given graphically in Chart III of the opsonic indexes.

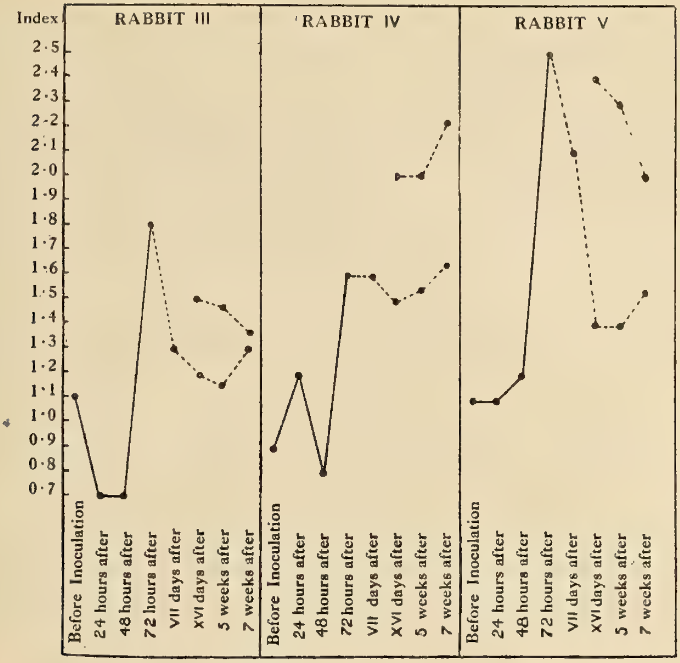

Chart III.—Staphylococcus Vaccine and Endotoxins.

Rabbit III received 1 c.c. (= 1000×10^6 = 0·1 milligramme) Staphylococcus Vaccine.
Rabbit IV received 0·1 milligramme Staphylococcus Endotoxin, *old* strain.
Rabbit V received 0·1 milligramme Staphylococcus Endotoxin, *new* strain.

Main trace = index determined with old strain of Staphylococcus.
Small upper trace = index determined with new strain of Staphylococcus.

It will be seen from these experiments that the endotoxin prepared from the old laboratory strain (Rabbit IV) gave nearly as marked a rise in the opsonic index as the vaccine (Rabbit III), but that the former seems to induce less negative phase than the latter, and its effect is more persistent. The endotoxin prepared from the recently isolated strain (Rabbit V) induced a rise in the opsonic index much more marked than that induced by either the vaccine or the endotoxin prepared from the old laboratory strain. The sera, some time after inoculation (two to seven weeks), tested against the recently isolated strain, gave an opsonic index slightly higher in the case of the vaccine (Rabbit III) and much higher in the case of the endotoxins (Rabbits IV and V) than that obtained when tested against the old laboratory strain.

The effects of different amounts (0·1, 0·01, 0·001 milligramme) of another freshly prepared staphylococcus endotoxin solution were also tested, and the results are given graphically in Chart IV of the opsonic indexes, and are there compared with the injection of an ordinary dose of staphylococcus vaccine (1·0 c.c. = 1000×10^6 cocci = 0·1 milligramme).

From this chart (IV) it will be seen that a marked rise in the opsonic index results from the injection of staphylococcus endotoxin, and that the rise corresponds with the dose of endotoxin given. Even the smallest dose of endotoxin (0·001 milligramme, Rabbit IX), produced a considerable and lasting rise in the index, a rise more marked than in the case of the vaccine (Rabbit VI).

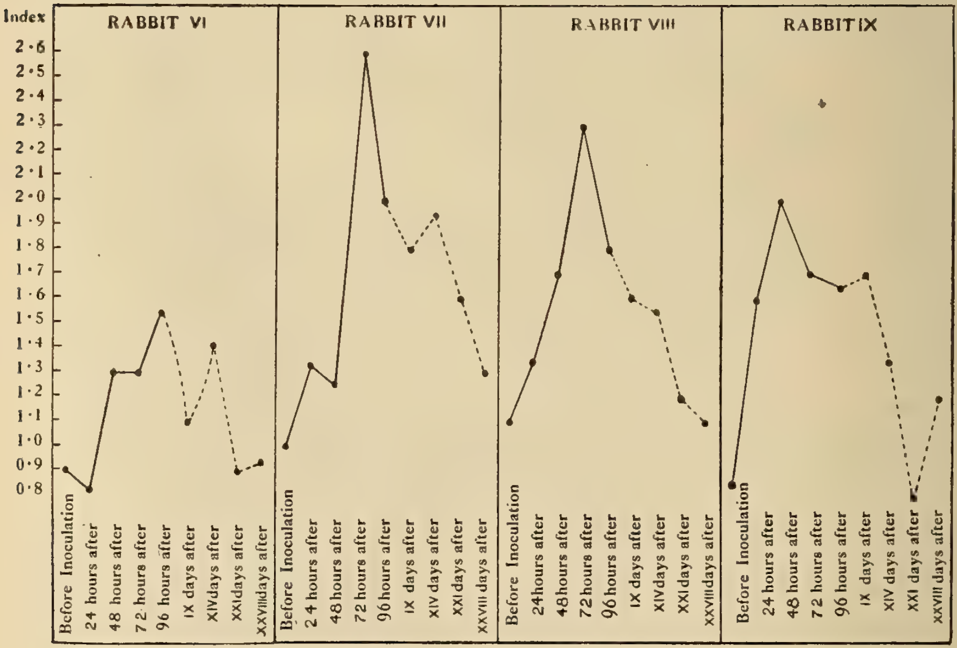

CHART IV.—Staphylococcus Vaccine and varying doses of Staphylococcus Endotoxin.

Rabbit VI received 1·0 c.c. (1000×10^6 cocci = 0·1 milligramme) Staphylococcus vaccine.
Rabbit VII received 0·1 milligramme Staphylococcus endotoxin.
Rabbit VIII received 0·01 milligramme Staphylococcus endotoxin.
Rabbit IX received 0·001 milligramme Staphylococcus endotoxin.

A few experiments on the effect of dilution on the opsonic action of a vaccine serum and of an endotoxin serum were also made, and the results obtained are given in Table II.

From Table II it will again be seen that the endotoxin produces a greater rise in the opsonic index than the vaccine does. In this case dilution does not affect the index in the same way as dilution of typhoid serum; on the whole the index remains much the same in the undiluted and the diluted serum, though with dilutions of 1 in 5 and 1 in 10 more cocci are ingested by the leucocytes than when the serum is undiluted, and this applies to the serum both of the inoculated, and of the uninoculated, animals.

Table II.—Effects of Dilution on Staphylococcus Vaccine and Endotoxin Sera. Number of Cocci ingested by 50 Polymorphonuclear Leucocytes.

Period.	Dilution of serum.	Control (opsonic index = 1).	Vaccine serum (1 c.c. vaccine).	Opsonic index.	Endotoxin serum (0·1 mgrm.).	Opsonic index.
Control before inoculation	undiluted	210	235	1·1	199	0·95
	1 in 5	335	356	1·06	315	0·94
	1 10	432	410	0·95	397	0·92
24 hours after inoculation	undiluted	233	183	0·8	400	1·3
	1 in 5	286	256	0·9	241	0·85
	1 10	295	190	0·64	214	0·7
	1 20	108	121	1·1	144	1·3
48 hours after inoculation	undiluted	85	131	1·5	326	3·8
	1 in 10	100	169	1·7	358	3·6
72 hours after inoculation	undiluted	58	92	1·6	156	2·7
	1 in 10	80	133	1·66	131	1·6
6 days after inoculation	undiluted	125	186	1·5	306	2·4
	1 in 10	185	211	1·14	365	2·0
12 days after inoculation	undiluted	102	131	1·3	177	1·75

(See Note at end of Table I.)

C. *Bacillus tuberculosis.*—Two preparations of tubercle endotoxin were used, one prepared from untreated tubercle bacilli, the other prepared from tubercle bacilli previously extracted with ether. The effect produced by the endotoxins was compared with that produced by a small dose of German Tuberculin R. The results obtained are charted graphically in Chart V of the opsonic indexes.

From Chart V it will be seen that the German Tuberculin R (dose 0·002 milligramme) produced little effect (Rabbit X). A corresponding dose of tubercle endotoxin (prepared with *unextracted* bacilli), on the other hand, induced a marked and prolonged rise in the opsonic index, which was preceded by a slight negative phase (Rabbit XI). A relatively huge dose of the same endotoxin (1 milligramme) produced a decided negative phase followed by a rise in the opsonic index of approximately the same amount as that produced by the smaller dose (Rabbit XII). A similar dose (1 milligramme) of the endotoxin prepared with ether-extracted tubercle bacilli produced an alteration (negative phase and subsequent rise) less marked than with the endotoxin prepared from the unextracted bacilli (Rabbit XIII).

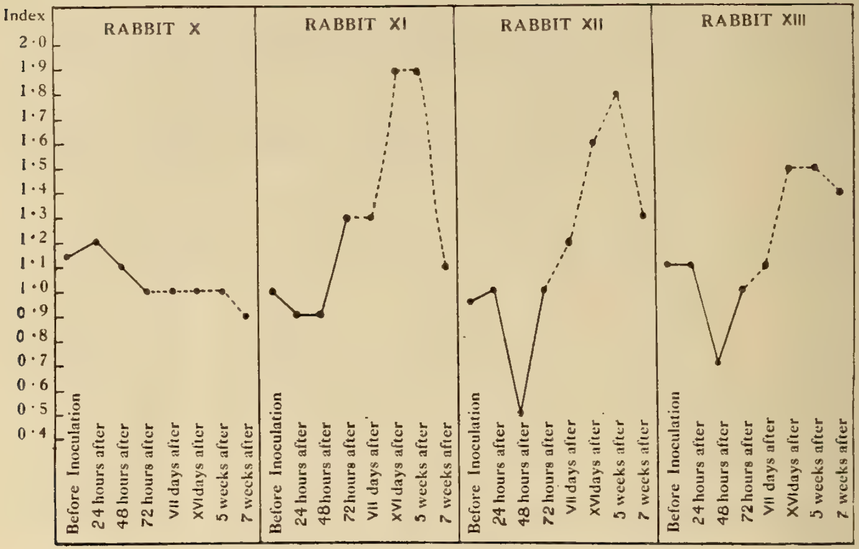

CHART V.—Tuberculin and Tubercle Endotoxin.

Rabbit X received 0·002 milligramme Tuberculin R.
Rabbit XI received 0·002 milligramme tubercle endotoxin.
Rabbit XII received 1·0 milligramme tubercle endotoxin.
Rabbit XIII received 1·0 milligramme ether-extracted tubercle endotoxin.

D. *Effect of keeping on the Activity of the Endotoxin Solutions.*—In view of the possible use of endotoxin solutions for vaccine treatment, it was thought desirable to make tests on their activity after they had been kept for a period. The tubercle endotoxin was prepared on March 9, 1908, and the staphylococcus endotoxin was prepared on March 10, 1908. They were the same solutions as those employed in the experiments detailed in Sections B and C above, were kept in an ice-safe and were injected on May 1, 1908, into fresh rabbits, *i.e.* approximately seven weeks after preparation. The results are given graphically in Charts VI and VII of the opsonic indexes.

In the case of the tubercle endotoxin, doses similar to those given in the experiments in Section C were administered. It will be seen from Chart VI of the opsonic indexes that the endotoxin solutions were quite as active as previously. Rabbit XV, receiving the large dose of endotoxin prepared from unextracted bacilli, became unwell on the twelfth day, and died on the thirty-sixth day, after injection.

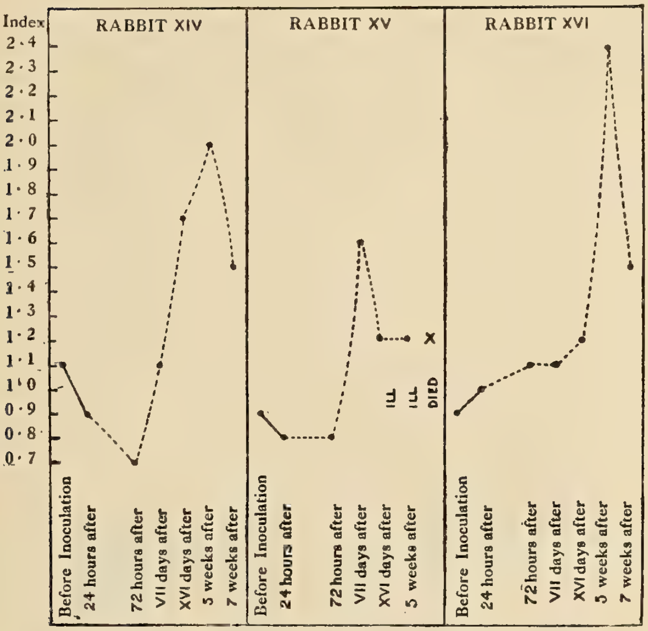

CHART VI.—*Old* Tubercle Endotoxin Solutions (7 weeks old).

Rabbit XIV received 0·002 milligramme tubercle endotoxin.
Rabbit XV received 1·0 milligramme tubercle endotoxin.
Rabbit XVI received 1·0 milligramme ether-extracted tubercle endotoxin.

In the case of the staphylococcus endotoxin, in view of possible deterioration on account of keeping, 10 times the dose (1 milligramme) previously given was injected. It will be seen from Chart VII of the opsonic indexes that a marked effect on the opsonic index was induced, greater, as before, in the case of the endotoxin prepared from the recently isolated strain (Rabbit XVIII).

Another experiment with the latter endotoxin (prepared on March 9, 1908) was performed on November 3, 1908, approximately eight months after preparation, the dose being 0·1 milligramme. Again it will be seen from Chart VIII of the opsonic indexes that a marked effect was produced (Rabbit XIX). The staphylococcus endotoxin employed in the experiment detailed in Section E below was the same preparation and was then 10 months old, and from Chart IX it will be seen that it was still very active.

These experiments indicate that the endotoxin solutions deteriorate but slowly, and retain a considerable proportion of their activity for at least three to six months.

E. *Production of "Negative Phase" by Injection of Endotoxin.*—It was

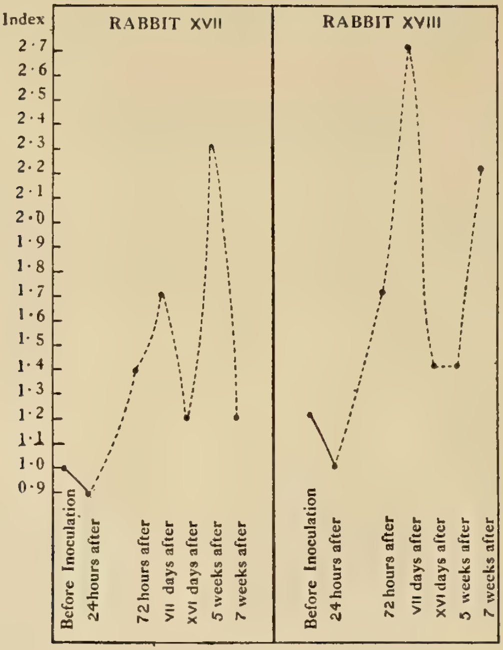

CHART VII.—*Old* Staphylococcus Endotoxin Solutions (7 weeks old).
Rabbit XVII received 1·0 milligramme Staphylococcus endotoxin, *old* strain.
Rabbit XVIII received 1·0 milligramme Staphylococcus endotoxin, *new* strain.

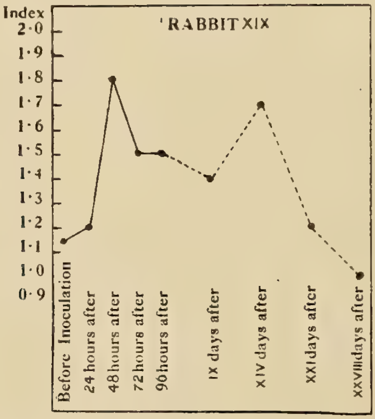

CHART VIII.—Old Staphylococcus Endotoxin Solution (8 months old). (Prepared from *new* strain of Staphylococcus.) Rabbit XIX received 0·1 milligramme endotoxin.

considered desirable to attempt to ascertain whether the endotoxin produces a negative phase comparable to that produced by a vaccine. For this purpose relatively large doses of staphylococcus vaccine ($15{,}000 \times 10^6$ cocci = 1·5 c.c. vaccine), and of fresh staphylococcus endotoxin (1 milligramme) were injected, and the opsonic index was determined 15 hours, 20 hours, 24 hours, 48 hours, and 72 hours, and five days and seven days after inoculation. The results are given graphically in Chart IX of the opsonic indexes.

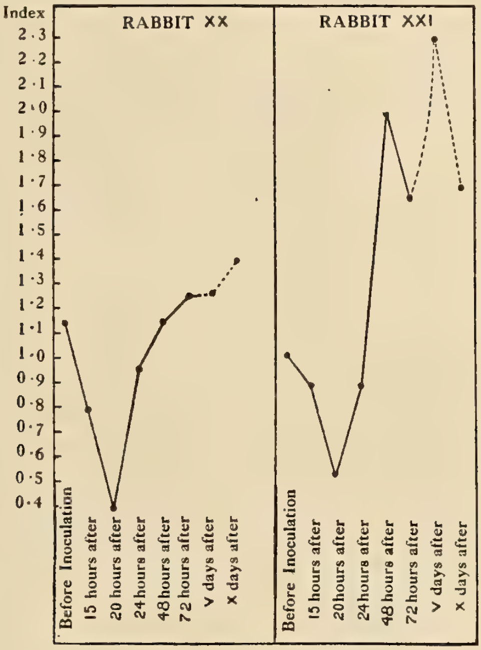

CHART IX.—To ascertain extent of "Negative Phase" with large doses of Staphylococcus Vaccine and Endotoxin.

Rabbit XX received 1·5 c.c. (= 15×10^6 cocci = 0·15 milligramme) vaccine.
Rabbit XXI received 1·0 milligramme endotoxin.

From these experiments (Chart IX) it would appear that the vaccine (Rabbit XX) produces a decidedly greater negative phase at the twentieth hour after injection than the endotoxin does (Rabbit XXI), although, weight for weight, six and a half times as much active material was administered in the case of the endotoxin than in that of the vaccine. The results of this experiment (and also of those detailed in Sections B, C, and D) suggest that the endotoxin induces decidedly less negative phase than a vaccine.

I have to express my best thanks to Mr. Wellcome for the facilities he has afforded me at the Wellcome Physiological Research Laboratories for carrying out the greater part of this work, and to Mr. E. Thompson, Laboratory Assistant, on whom much of the labour of making the counts of the opsonic determinations has fallen.

(Part of the expense incurred in this work has been defrayed by a grant from the British Medical Association.)

The Alcoholic Ferment of Yeast-juice. Part IV.—*The Fermentation of Glucose, Mannose, and Fructose by Yeast-juice.*

By Arthur Harden, F.R.S., and W. J. Young.

(Received June 10,—Read June 24, 1909.)

The results previously communicated by the authors[*] were obtained exclusively with glucose, and in the present paper an account is given of the behaviour of mannose and fructose towards yeast-juice both in the presence and absence of added phosphate.

Buchner examined the fermentation of fructose by yeast-juice and found that it proceeded at precisely the same rate as that of glucose.[†] No experiments with mannose appear to have been previously performed.

The fructose employed throughout these experiments was Kahlbaum's crystallised fructose prepared from inulin. The mannose was prepared by the hydrolysis of ivory-nut and was purified by conversion into the phenylhydrazone, which was recrystallised from hot water and was finally decomposed by benzaldehyde in the usual manner.[‡] All the experiments were performed at 25° in the presence of toluene.

I. *Relative Rates of Fermentation of Glucose, Mannose, and Fructose.*

Both mannose and fructose are freely fermented by yeast-juice, as they are also by living yeast. The relative rates of fermentation of these three sugars by yeast-juice vary somewhat in different experiments, but on the average of the experiments performed the fructose appears to be fermented rather more quickly than either mannose or glucose, whilst the mannose is also fermented slightly more rapidly than glucose.

Table I gives the experimental results, 25 c.c. of yeast-juice being employed in each case.

In experiments 1, 2, and 4 the rates were taken after the mixture had been incubated for about half an hour. In experiment 3, observations of rate were made during three different intervals, and the time during which the mixture had been incubated before the commencement of each of these was—(*a*) 25 minutes, (*b*) 70 minutes, (*c*) 130 minutes.

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[*] 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 405; 1908, vol. 80, p. 299.

[†] 'Die Zymasegärung,' p. 100.

[‡] Herzfeld, 'Ber. Deutsch. Chem. Ges.,' 1895, vol. 28, p. 440.

Table I.—Relative Rates of Fermentation of Glucose, Mannose, and Fructose by Yeast-juice.

No. of exp.	Amount of sugar.	Total volume.	Time in mins.	Rate of fermentation. Cubic centimetres evolved in the time given.			Ratio of rates.		
				Glucose.	Mannose.	Fructose.	Glucose.	Mannose.	Fructose.
1	1	25·6	45	3·2	3	5·3	1	0·94	1·66
2	1	25·6	60	24·8	28	29	1	1·13	1·17
3*a*	4	45	35	6·8	7·2	8·7	1	1·06	1·28
b	4	45	60	10·4	11	14·1	1	1·06	1·36
c	4	45	265	40·2	38·6	46·9	1	0·96	1·17
4	1·5	32·5	135	51·8	55·9	55·7	1	1·08	1·08

II. *Total Fermentation.*

The total weight of carbon dioxide evolved from an excess of the sugar by a given volume of yeast-juice was also found to be slightly greater with fructose than with glucose, whilst that evolved from mannose in the only two experiments made was considerably less than from glucose.

The following are the experimental results, 25 c.c. of yeast-juice being employed, and the incubation continued until fermentation had ceased:—

Table II.—Total Fermentation of Glucose, Mannose, and Fructose.

No. of exp.	Sugar added.	Total volume.	Total carbon dioxide in grammes.			Ratio of totals.		
			Glucose.	Mannose.	Fructose.	Glucose.	Mannose.	Fructose.
5	4	32·5	0·6556	0·4452	0·7436	1	0·68	1·13
6	4	32·5	0·7405	0·6226	0·8624	1	0·67	1·16

III. *Fermentation of Mannose by Yeast-juice in presence of Phosphate.*

Mannose behaves towards phosphates in the presence of yeast-juice in precisely the same manner as glucose.* A rapid rise in the rate of fermentation occurs; an extra amount of carbon dioxide and alcohol are produced which are equivalent to the phosphate added, and the phosphate is converted into a hexosephosphate which is not precipitable by magnesium citrate mixture and can be isolated in the form of a lead salt. As in the case of glucose, an optimum concentration of phosphate exists at which a maximum rate of fermentation occurs. Beyond this optimum, increase of concentration

* Harden and Young, *loc. cit.*

of phosphate lowers the rate of fermentation. The rates obtained with mannose and glucose in comparative experiments are approximately equal.

These phenomena are illustrated by the following experiments:—

Experiment 7.—Two quantities of 25 c.c. of yeast-juice + 5 c.c. of a solution containing 1 gramme of the sugar were incubated until a constant rate had been attained, and 5 c.c. of an approximately 0·3 molar solution of sodium phosphate were then added.

Experiment 8.—Two quantities of 25 c.c. of a different sample of yeast-juice + 1 gramme of the sugar were incubated as above and 10 c.c. of the same sodium phosphate solution (0·3 molar) were added.

The readings made after the addition of the phosphate are tabulated below, the numbers expressing the volume of carbon dioxide evolved in the five minutes preceding the time given in the first column:—

Table III.—Fermentation of Glucose and Mannose in presence of Phosphate.

Time after addition.	Carbon dioxide evolved in preceding 5 minutes.			
	Experiment 7.		Experiment 8.	
	Glucose.	Mannose.	Glucose.	Mannose.
5	4·4	6·4	22·4	22·5
10	8·2	6·0	23·8	26·7
15	9·1	6·8	22·2	22·1
20	9·6	7·0	16·8	14·5
25	7·9	6·0		
30	4·0	4·8		
35	1·5	2·7		
40	1·0	1·0		
45	1·1	1·0		

Experiment 9. *Formation of a Hexosephosphate.*—Twenty-five cubic centimetres of yeast-juice were incubated until the rate of fermentation became constant at 0·8 c.c. in five minutes. Ten cubic centimetres of a 0·3 molar solution of sodium phosphate were then added. The rate rose to 7·1 c.c. in five minutes, and incubation was continued until it had again fallen. The liquid was then boiled and filtered, and the amount of free phosphate estimated as $Mg_2P_2O_7$. The whole solution was found to yield 0·0567 gramme of $Mg_2P_2O_7$. The phosphate added corresponded to 0·3263 gramme $Mg_2P_2O_7$, and hence the difference between these quantities, $0·3263 - 0·0567 = 0·2696$, corresponds to the minimum amount of phosphate rendered non-precipitable by magnesium citrate mixture.

Experiment 10. *Equivalence of extra Carbon Dioxide evolved to the*

Phosphate added.—Parallel experiments with glucose, mannose, and fructose were made with yeast-juice from the same preparation, the method being that previously described.* Three quantities of 25 c.c. of yeast-juice + 5 c.c. of a solution containing 1 gramme of the sugar to be examined were incubated with toluene at 25° for one hour, and to each were then added 5 c.c. of a solution of sodium phosphate corresponding to 0·1632 gramme of $Mg_2P_2O_7$ and equivalent to 32·6 c.c. of carbon dioxide at N.T.P. The rates of fermentation were then observed until they had again fallen and attained a steady value, the gases being measured moist at 19°·3 and 760·15 mm.

Table IV.—Relation of Carbon Dioxide evolved to Phosphate added for Glucose, Mannose, and Fructose.

	Glucose.	Mannose.	Fructose.
Maximum attained	9·6	7	11·3
Final rate of juice, cubic centimetres in 5 minutes	1·1	0·96	1·08
Total evolved in 55 minutes after addition of phosphate	49·7	47·8	47·6
Correction for rate of juice in absence of phosphate	12·1	10·6	11·9
Total equivalent to phosphate	37·6	37·2	35·7
,, ,, at N.T.P.	34·4	34·0	32·6

These numbers agree well with the value calculated from the phosphate added, viz., 32·6 c.c.

Experiment 11. *Effect of an Excess of Phosphate.*—Two quantities of 15 c.c. of yeast-juice + 7 c.c. of a solution of mannose containing 1 gramme of the sugar were treated with 10 and 15 c.c. respectively of a 0·6 molar solution of potassium phosphate, K_2HPO_4. The following readings were obtained, showing that in presence of 15 c.c. of the phosphate solution the rate is less than in presence of 10 c.c., and that in neither case is a high maximum rate attained:—

Table V.—Effect of an Excess of Phosphate on the Fermentation of Mannose.

Time after addition in minutes.	Rate in preceding 5 minutes.	
	10 c.c. phosphate.	15 c.c. phosphate.
10	2·2	2·1
20	1·9	1·6
30	2·4	1·4
40	3·0	1·5
60	4·2	1·7

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* 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 414.

IV. *Fermentation of Fructose by Yeast-juice in the presence of Phosphate.*

Fructose, like mannose, agrees qualitatively with glucose in its behaviour towards phosphates, but it differs quantitatively from both these sugars in two important respects: (1) The optimum concentration of phosphate is much greater; (2) the maximum rate of fermentation attainable is much higher.

These points of resemblance and dissimilarity are brought out by the following experiments:—

(*a*) When a phosphate is added to yeast-juice containing fructose the rate of fermentation rises to a maximum and then falls to a rate which is usually slightly higher than the original rate of fermentation.

Experiment 12.—Ten cubic centimetres of a 0·3 molar solution of sodium phosphate were added to a mixture of 25 c.c. of yeast-juice and 1 gramme of fructose, the original rate of fermentation of which was 0·8 c.c. in five minutes. The total volume of the mixture was 35·6 c.c. The readings were as follows:—

Table VI.—Fermentation of Fructose in presence of Phosphate.

Time after addition of phosphate in minutes.	Cubic centimetre of CO_2 evolved in preceding 5 minutes.
5	14·9
10	21·3
15	21·7
20	17·3
25	10·9
30	2·0
35	1·5
40	1·0
45	1·2

The maximum rate attained varies very considerably with different samples of yeast-juice, as is shown by the following numbers (see Table VII), which refer in each case to 25 c.c. of yeast-juice.

It is interesting to note that the two high rates, 80 and 76·2 c.c. per five minutes, are equal to about half the rate obtainable with an amount of living yeast corresponding to 25 c.c. of yeast-juice, assuming that about 40 grammes of yeast are required to yield this amount of juice, and that this amount of yeast would give about 140 c.c. of carbon dioxide per five minutes at 25°, which has been found experimentally to be the average rate obtainable with the top yeast employed for these experiments.

Table VII.—Maxima attained by the Fermentation of Fructose in presence of Phosphate.

Number of experiment.	Volume of 0·6 molar phosphate solution added.	Total volume.	Maximum rate attained, cubic centimetres CO_2 in 5 minutes.
	c.c.	c.c.	
13	12·5	75	80
14	12·5	50	27·1
15	10	50	31·2
16	20	55	76·2

(*b*) Equivalence of the extra carbon dioxide evolved to the phosphate added. One example of this has already been given in Experiment 10, p. 338.

Experiment 17.—In another case the phosphate added was equivalent to 65·2 c.c. of CO_2 at N.T.P.

	c.c.
Gas evolved in 45 minutes	82·2
Correction for rate of juice 1·2 c.c. per 5 minutes for a period of 45 minutes	10·8
Gas evolved at 19 and 747·75	71·4
Volume at N.T.P.	64·2

(*c*) Production of a hexosephosphate non-precipitable by magnesium citrate mixture.

Experiment 18.—The experiment was carried out precisely as Experiment 9. The amounts of phosphate are expressed as $Mg_2P_2O_7$:—

Phosphate added	0·3263
Free phosphate after fermentation	0·0426
Phosphate rendered non-precipitable............	0·2837

The solution after boiling was found to contain a hexosephosphate which has been isolated in the form of a lead salt and is at present undergoing investigation.

(*d*) Existence of an optimum concentration of phosphate.

The following table shows the maximum rates produced by the addition of varying volumes of a 0·6 molar solution of potassium phosphate, K_2HPO_4, to yeast-juice and fructose. In all comparable experiments the total volumes were kept equal by the addition of a solution of potassium bicarbonate as previously explained for glucose;* in each case 2 grammes of fructose were employed. The maximum obtained and the optimum concentration are printed in thick type:—

Table VIII.—Maximum Rates of Fermentation and Optimum Concentrations of Phosphate for Fructose.

No. of experiment.	Volume of yeast-juice.	Total volume.	Cubic centimetres of 0·6 molar solution of K_2HPO_4 added.	Maximum rate per 5 minutes.
	c.c.	c.c.		c.c.
19*a*	5	25	0	0·5
b	5	25	**5**	**14·2**
c	5	25	10	5·5
d	5	25	15	1·8
20*a*	15	40	3	22·5
b	15	40	**7·5**	**25·4**
c	15	40	10	20·7
d	15	40	15	11·3
e	15	40	20	7·4
21*a*	10	35	3	31·5
b	10	35	**5**	**32·2**
c	10	35	7·5	28·5
d	10	35	10	20·2
e	10	35	15	9·2
f	10	35	20	5·7

It thus appears that, precisely as in the case of glucose, progressive increase in the concentration of phosphate beyond the optimum produces a corresponding decrease in the rate of fermentation, and at a high concentration the rate becomes extremely slow.

(*e*) Comparison of the optimum concentrations of phosphate and of the maximum rates produced at those concentrations for fructose and glucose.

The following results, which all refer to 10 c.c. of yeast-juice, clearly show that the optimum concentration of phosphate for the fermentation of fructose is from 1·5 to 10 times that for glucose, and that the maximum rate of fermentation for fructose is two to six times that of glucose.

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* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 307.

Table IX.—Optimum Concentrations of Phosphate and Maximum Rates of Fermentation for Fructose and Glucose.

No. of experiment.	Sugar, in grammes.	Total volume.	Optimum volume of 0·6 molar phosphate.		Maximum rate in cubic centimetres of CO_2 per 5 minutes.	
			Glucose.	Fructose.	Glucose.	Fructose.
22	2	35	2	5	7·5	32·2
23	4	50	1	10	5·4	28·4
24	1·6	23	2	5	8	17
25	1	25	1·75	5	5·2	25·9
26	2	25	5	7·5	16·2	31·2
27	2	20	2	3·5	7·9	22·6
28	2	22·5	0·75	2	3·4	22·2

V. *Effect of the Addition of Fructose on the Fermentation of Glucose or Mannose in presence of a large Excess of Phosphate.*

When the rate of fermentation of glucose or mannose by yeast-juice is greatly lowered by the presence of a large excess of phosphate, the addition of a relatively small amount of fructose causes rapid fermentation to occur. This induced activity is not due solely to the fermentation of the added fructose, since the amount of this sugar may be insufficient to yield the gas evolved.

The general nature of this phenomenon may be gathered from the following experiment:—

Experiment 29.—Two quantities of 25 c.c. of yeast-juice + 2 grammes glucose + 20 c.c. of 0·6 molar K_2HPO_4 solution + toluene were incubated at 25°. The amount of phosphate was largely in excess of the optimum, and the rate of fermentation was found to be 1·8 c.c. per five minutes.

A. To one of these were added 1 c.c. of glucose solution containing 0·2 gramme of the sugar, and 4 c.c. of the phosphate solution. The rate of fermentation fell to 1·5 and continued at this value.

B. To the other were added 1 c.c. of a solution containing 0·2 gramme of fructose and 4 c.c. of the phosphate solution. The rate of fermentation at once rose, as shown by the following readings.

As the evolution of carbon dioxide proceeds the phosphate is converted into hexosephosphate, and its effect on the fermentation of the glucose lessened, and hence in order to maintain the original concentration of phosphate it is necessary to add a fresh quantity at intervals to B, the amount required being calculated from the gas evolved, 13·5 c.c. of CO_2 at

Time after addition.	CO₂ evolved in preceding 5 minutes.	
	Substances added.	
	A. Glucose and phosphate.	B. Fructose and phosphate.
5 minutes	1·5	2·6
10 "	1·8	6·9
15 "	1·2	13·8
20 "	1·5	19·4
25 "	1·5	25·1

17°·2 and 762·4 mm. being equivalent to 2 c.c. of the phosphate solution. The further course of the experiment was as follows:—

B.

Time after addition of phosphate.	Gas evolved since last addition of phosphate.	Phosphate added 0·6 molar solution.	Rate per 5 minutes.
minutes.	c.c.	c.c.	c.c.
25	67·8	4	25·1
30	—	—	27·1
35	53·9	10	26·8
40	—	—	22·1
45	—	—	19·7
50	—	—	18·5
55	—	—	19·8
60	—	—	20·5
65	120·5	10	19·9
70	—	—	17·3
75	—	—	18·2
80	—	—	17·4
85	—	—	15·8
90	—	—	16·8
95	97·9	—	12·4
Total gas evolved	340·1		

Hence, although the concentration of the phosphate was never allowed to fall much below the original value and was generally considerably above it, the addition of 0·2 gramme of fructose to 2 grammes of glucose produced a total evolution of 340·1 c.c. of CO_2, corresponding to the total fermentation of 1·3 grammes of sugar (in the ratio $C_6H_{12}O_6 : 2\,CO_2$), whereas in the absence of fructose the total fermentation would have been only 28·5 c.c., corresponding to 0·22 gramme of sugar. As the fructose added was only 0·2 gramme and the subsequent evolution of carbon dioxide corresponded to the total fermentation of 1·3 grammes of sugar, it is obvious that the addition of the fructose must have induced the fermentation of the glucose.

Experiment 30.—Similar results were obtained in another way by employing so large an excess of phosphate that the fermentation observed did not reduce the concentration of phosphate to the limit at which the rapid fermentation of glucose in the absence of added fructose became possible.

Four quantities of 15 c.c. of yeast-juice + 5 c.c. of a solution containing 2 grammes of glucose were employed—

1. 5 c.c. of 0·6 molar potassium phosphate solution were added.
2. 7·5 c.c. of phosphate solution were added.
3. 15 c.c. of phosphate solution were added.
4. 15 c.c. of phosphate solution and 0·5 c.c. of a solution containing 0·05 gramme of fructose were added.

The following observations were then made :—

Time.	Rate for 5 minutes.			
	1.	2.	3.	4.
5 minutes	2·0	1·9	0·8	4·2
10 "	2·6	1·6	1·1	4·0
15 "	3·7	2·2	0·6	6·6
20 "	3·7	2·1	0·8	8·9
25 "	5·2	2·6	0·7	11·6
30 "	6·4	2·9	0·6	14·2
35 "	9·6	3·5	0·7	14·8
Total evolved	33·2	16·8	5·3	64·3

In this case the amount of gas evolved in No. 4 is equivalent only to 4·8 c.c. of the phosphate solution, and the final concentration is therefore 10·2 c.c. of phosphate in 35 c.c. The concentration of phosphate, therefore, never falls as low as that present in No. 2 (7·5 in 27·5 or 9·5 in 35), and yet the fermentation is much more rapid than in this flask, which itself contains a concentration of phosphate greatly in excess of the optimum, as shown by a comparison with No. 1. The amount of carbon dioxide yielded by the complete fermentation of 0·05 gramme of fructose is only 13·5 c.c., so that there can be no doubt that most of the gas evolved was derived from the glucose.

(Note.—Experiments 1, 2, and 4 are not strictly comparable, since the contents of the flasks were not made up to the same volume, but the difference in rate due to this is negligible.)

VI. *Specific Character of the Inductive Action of Fructose.*

This inductive effect is specific to fructose and is not produced when glucose is added to mannose or fructose, or by mannose when added to glucose or fructose, under the proper conditions of concentration of phosphate in each case.

The experiments on this point were carried out by ascertaining in each case the excess of phosphate necessary to produce a slow rate of fermentation and then making two parallel experiments, one with, and the other without, the addition of the small quantity of the sugar to be tested for inductive power. All the observations were made with 15 c.c. of yeast-juice.

Table X.—Specific Character of the Inductive Effect of Fructose.

No. of experiment.	Cubic centimetres 0·6 molar phosphate.	Total volume.	Grammes of sugar present.			Gas evolved.	Time.
			Mannose.	Fructose.	Glucose.		
		c.c.				c.c.	mins.
31*a*	15	37	1	0	0	8·7	30
b	15	37	1	0·1	0	43·3	30
c	15	37	1	0	0·1	10·9	30
32*a*	10	32	1	0	0	18·9	30
b	10	32	1	0·1	0	76·3	30
33*a*	60	82·5	0	1	0	13·6	20
b	60	82·5	0·15	1	0	7·9	20
34*a*	10	30	0	0	1	11·4	30
b	10	30	0·1	0	1	14·1	30
35*a*	75	90	0	2	0	18·4	30
b	75	90	0	2	0·1	16·1	30

This remarkable property of fructose, taken in connection with the facts that this sugar in presence of phosphate is much more rapidly fermented than glucose or mannose, and that the optimum concentration of phosphate for fructose is much higher than for glucose or mannose, appears to indicate that fructose when added to yeast-juice does not merely act as a substance to be fermented, but, in addition, bears some specific relation to the fermenting complex.

All the phenomena observed are, indeed, consistent with the supposition that fructose actually forms a permanent part of the fermenting complex, and that, when the concentration of this sugar in the yeast-juice is increased, a greater quantity of the complex is formed. As the result of this increase in the concentration of the active catalytic agent, the yeast-juice would be capable of bringing about the reaction with sugar in presence of phosphate at a higher rate, and at the same time the optimum concentration of phosphate would become greater, exactly as is observed. The question whether, as suggested above, fructose actually forms part of the fermenting complex, and the further questions, whether, if so, it is an essential constituent, or whether it can be replaced by glucose or mannose with formation of a less active complex, remain at present undecided, and cannot profitably be more fully discussed until the results of experiments now in progress are available.

It must, moreover, be remembered that different samples of yeast-juice vary to a considerable extent in their relative behaviour to glucose and fructose, so that the phenomena under discussion may be expected to vary with the nature and past history of the yeast employed.

Summary.

1. Mannose behaves towards yeast-juice both in the presence and in the absence of added phosphates in the same manner as glucose.

2. Fructose resembles both glucose and mannose in its behaviour towards yeast-juice, but in the presence of phosphates is much more rapidly fermented than the other sugars, and the optimum concentration of phosphate is much higher.

3. Fructose has the property of inducing rapid fermentation in presence of yeast-juice in solutions of glucose and mannose containing such an excess of phosphate that fermentation is only proceeding very slowly. No similar property is possessed by glucose or mannose.

The Discovery of a Remedy for Malignant Jaundice in the Dog and for Redwater in Cattle.

By George H. F. Nuttall, M.D., Ph.D., Sc.D., F.R.S., Quick Professor of Biology in the University of Cambridge; and Seymour Hadwen, D.V.Sci. (McGill) of the Department of Agriculture, Canada.

(Received June 22,—Read June 24, 1909.)

Judging from the literature relating to piroplasmosis, no drug is known which exerts any curative action on either canine or bovine piroplasmosis. The canine disease is exceedingly fatal, and, in certain localities, especially in South Africa, it is almost impossible to keep dogs. On the other hand, the disease in cattle causes enormous financial losses, especially in America, Australia, and Africa, not only by causing a considerable mortality, but also by producing a long-lasting anæmia in many of the affected animals.

The discovery of a drug which will bring about a cure of piroplasmosis is therefore a matter of practical importance. Our object in publishing this communication is to announce the discovery of such a remedy. Whilst a full description of our experiments will shortly be published in 'Parasitology,' we desire to place the main facts on record.

Canine Piroplasmosis.

We have discovered that trypanblau and trypanrot are highly efficient remedies in the treatment of canine piroplasmosis. The drugs exert a direct and observable effect upon the parasites by causing the pyriform parasites to quickly disappear; in most cases causing the total disappearance of the parasites from microscopic observation in the peripheral blood. The disappearance of the parasite is usually temporary, since they may reappear in small numbers after an interval of 9 to 12 days; the treated dogs, as a rule, show no symptoms and gradually progress towards complete recovery. In our experience the treated dogs show little or no loss of weight; this being in marked contrast to what is usually observed in the dogs which chance to recover naturally.

Our experiments were carried out upon 20 dogs of various breeds and ages, the majority being highly susceptible puppies. We experimented with a very virulent strain of *Piroplasma canis* from Cape Colony. All of the 7 control (untreated) dogs died of piroplasmosis: 6 died within 7 to 13 days, and 1 on the 36th day after inoculation with virulent blood.

The remaining dogs, 13 in number, were treated as follows:—

Results of Treatment with Trypanblau (up to June 17, 1909).

Dog.	Treated dogs.	Control dogs. (Inoculated at the same time as the treated dogs.)
1	Kept under observation 90 days......................	Control died after 7 days.
2	,, ,, 83 ,, ...	,, ,, 7 ,,
3	,, ,, 69 ,, ...	,, ,, 9 ,,
4	,, ,, 65 ,, ...	,, ,, 8 ,,
5	,, ,, 42 ,, ...	,, ,, 36 ,,
6	,, ,, 43 ,, ...	,, ,, 8 ,,
7	,, ,, 52 ,, ...	,, ,, 7 ,,
8	Died of relapse after 13 ,, ...	,, ,, 7 ,,
9	,, ,, 15 ,, ...	,, ,, 7 ,,
10	Treated when moribund, the dog died three hours later, but the drug obviously affected the parasites	
11	Treated 24 hours after inoculation (*i.e.* before the parasites had appeared), dog alive and well after 65 days; it never showed parasites	,, ,, 7 ,,

Results of Treatment with Trypanrot.

Dog.	Treated dogs.	Control dogs.
12	Alive and well after 111 days	Control dog died after 9 days.
13	Treated in an advanced stage of the disease, the dog died 20 days after inoculation, but no parasites could be found in the blood. The dog apparently died from the after effects of piroplasmosis	,, ,, 7 ,,

The above table speaks for itself, it scarcely requires any comment. We very much regret that we have lost four of our treated dogs from inter-current disease. Two of these dogs died of distemper (42 and 43 days respectively after inoculation), one died of distemper and mange combined (69 days after inoculation), and one died of severe generalised mange due to *Demodex folliculorum* (52 days after inoculation). Dog 13 was treated in an advanced stage of the disease, but its life was prolonged; it is worthy of note that no parasites could be found in the animal at autopsy. Dog 10 was treated only three hours before death; the drug markedly affected the parasites, but it was too late to save the dog's life. In only two cases did we see a relapse follow five days after apparently successful treatment; this was in two very small and poorly developed puppies. The remaining dogs are alive and well to-day and show no parasites microscopically in their blood.

As previously stated, the drugs exert a direct effect upon the parasites. The percentage of infected corpuscles is decreased, the pyriform parasites disappear, rounded and degenerated parasites are seen for a time, and, after a while, all parasites are lost to view. When, after an interval, the parasites reappear, they do so in exceedingly small numbers, and, after a while, they disappear completely and finally.

All of our dogs were treated by subcutaneous injections of saturated solutions of the dyes.

Bovine Piroplasmosis.

With regard to redwater, we are in a position to state that trypanblau exerts a very prompt effect upon the parasite. The effect is precisely similar to that observed in *Piroplasma canis.* Our experiments upon the bovine disease are still in progress, but we feel that they are sufficiently advanced to warrant the trial of the remedy in the field. We shall report upon our results in a future communication.

The results of these experiments are of considerable interest, since they throw additional light upon the biology of the parasites and entirely confirm the observations of Nuttall and Graham-Smith upon the usual mode of multiplication of the parasites in the circulating blood. The striking effects of the drugs upon the parasites led us directly to make enumerations of the different forms of parasites occurring in the blood of treated and untreated animals. The result of these observations has been to bring to light several interesting facts regarding the life-history of the parasites.

East Coast Fever.

Incidentally we may mention that in one experiment which we have tried, trypanblau exerted no effect whatsoever on the parasite of East Coast Fever in cattle. This does not appear to us surprising, since the parasite is very different from *Piroplasma,* although most writers still persist in retaining it in the genus. For reasons stated elsewhere by Nuttall (1908), the parasite of East Coast Fever should be named *Theileria parva.*

Conclusions.

The obvious practical conclusions to be drawn from our results is that the remedies will prove of value in practice. It is highly probable that they will act in a similar manner in relation to equine and ovine piroplasmosis. The mere fact that remedial agents have been found for diseases which have hitherto run their course, in spite of all treatment, is encouraging, since with time we may reasonably hope to cope with these maladies in an efficient manner.

The Vacuolation of the Blood-platelets: an Experimental Proof of their Cellular Nature.

By H. C. Ross, late Surgeon Royal Navy.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received May 12, 1909.)

On July 27, 1907, a paper appeared in the 'Lancet' by Ronald Ross, S. Moore, and C. E. Walker, entitled "A New Microscopical Diagnostic Method and some Simple Methods for Staining Liquid Blood." It described new methods for the staining of blood-cells *in vitro*—notably the agar-jelly method—by mixing polychrome methylene blue with agar and preparing a film: blood-cells spread upon this will absorb the stain, and if the jelly is suitably prepared the different morphological elements of the cells can be readily distinguished. It also described how the leucocytes, after they had been resting on the jelly for a short time, developed bright red spots in their cytoplasm; and it was suggested that the spots might be centrosomes.

The spots appear as bright scarlet points, and resemble closely what one would imagine that centrosomes would look like if they could be seen in polymorphonuclear leucocytes. The "red spots," however, could not be found in the blood-platelets, and believing them to be centrosomes, the authors suggested in their paper in the 'Lancet' that if they could be demonstrated in the blood-platelets, it would settle the nature of these bodies, and close an old existing controversy.

About two months ago I was fortunate enough to observe these "red spots" in the blood-platelets, and if the following experiment is repeated the spots can be produced in them in every specimen.

Prepare a neutral solution which contains 3 per cent. sodium citrate, 1 per cent. sodium chloride, and 1 per cent. morphine hydrochloride. Draw up into a capillary tube a volume of this solution, and add to it an equal volume of blood drawn freshly from the finger. After allowing the two fluids to mix, incubate the tube at 37° C. for five hours. A film of agar-jelly must now be prepared, which contains a sufficiency of Unna's stain to stain deeply the granules of polymorphonuclear leucocytes when they are spread upon it. One suitable formula for preparing this jelly is given in the 'Lancet' for January 16 and February 6, 1909, and another suitable formula will be found in the first equation in a paper recently published in the 'Proceedings of the Royal Society,' B, vol. 81, 1909, p. 102. Place a drop of the incubated blood and morphine solution on to a cover glass, which should be inverted and allowed to fall flat on to the agar film. In about

15 minutes' time it will be seen that probably all the blood-platelets will show one or more bright red spots in them, and these spots are identical in appearance (except that they are usually smaller) with those seen in the cytoplasm of leucocytes when blood is examined by this *in vitro* method by which the spots in the larger cells were observed by R. Ross, Moore, and Walker.

It is, however, necessary to state that the spots are not centrosomes. At first I believed them to be such, but further investigation showed the suggestion to be erroneous. They are diffusion vacuoles. If the diffusion of stain into leucocytes is accelerated by heat or alkali so as to cause maximum staining without actually killing the cells, then stained liquid also passes into the colloid cytoplasm and remains suspended in it as a "red spot." A description of the nature of these spots was published in the 'Journal of Physiology,' September, 1908. These vacuoles get gradually larger, and when the leucocytes die, the spots suddenly disperse owing to the liquefaction of the cytoplasm.

The suggestion of R. Ross, Moore, and Walker, that if the spots could be seen in the blood-platelets it would be proof of their cellular nature, was evidently based on the assumption that the spots were centrosomes. Since they are not centrosomes, it may be said that their appearance in the blood-platelets now falls into insignificance. But the spots in the platelets also get larger and then disperse after an interval, and their disappearance is practically coincident with the disappearance of the vacuoles in the leucocytes, which is caused by the liquefaction of the cytoplasm occurring at death. Morphia also causes extreme vacuolation of the leucocytes.

As a matter of fact, studying the question from another aspect also shows that their vacuolation is proof that the blood-platelets are cells. When fresh blood is placed on the jelly, and no morphia is employed, the leucocytes do not become extremely vacuolated, nor do the platelets develop the spots. Why should morphia have this effect ?

It was shown in a former paper* that different cells have different coefficients of diffusion, and that, in blood-cells, approaching death causes a lowered coefficient. It is obvious that the vacuoles will appear more readily, that is, liquid will diffuse more readily into cells which have a lowered coefficient of diffusion.

It is apparent, therefore, that the blood-platelets must be cells, or rather must be composed of living protoplasm, for their vacuolation is produced experimentally by the action of the poison, as morphia, in causing the gradual approach of death, also lowers the coefficient of diffusion. Morphia lowers the coefficient more than any substance that has been tried as yet, and it appears to have a greater effect on the cytoplasm than other poisons. As all the platelets in a specimen usually become vacuolated, I believe that they all belong to one class of cell, derived from one source.

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* 'Roy. Soc. Proc.,' B, vol. 81, 1909.

In view of the vacuolation, the precipitate theory of the blood-platelets becomes untenable. Another theory, that they are the extruded nuclei of red cells, can also, I think, be dimissed. Former researches have shown that the nucleus of a cell is its "higher centre." It is difficult to believe that a cell can thrust out its nucleus. Moreover, in the paper in 'The Journal of Physiology,' already referred to, it was stated that the spots in leucocytes were never seen to be connected with the nucleus. The platelets, therefore, can hardly be nuclei. The nuclear theory is, I believe, the outcome of the examination of dead structures. Blood-platelets are never seen emerging from red-cells when *in vitro* methods are employed.

I believe that the theory that the blood-platelets are fragments of leucocytes is the correct one; they have the same coefficient of diffusion as those cells, and usually contain a few granules which have the same staining rate as those of the leucocytes. When blood is placed on jelly which excites amœboid movement in leucocytes,* the platelets have been seen to extrude and retract pseudopodia. On a few occasions, a pseudopodium has been seen to become separated, and the fragment appears to be identical with the other platelets seen in the specimen. It may contain clear cytoplasm, a few granules, or even a lobe of the nucleus.

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* 'The Lancet,' January 16, 1909.

Further Results of the Experimental Treatment of Trypanosomiasis: being a Progress Report to a Committee of the Royal Society.

By H. G. Plimmer, F.L.S., and W. B. Fry, Captain R.A.M.C.

(Communicated by J. Rose Bradford, M.D., Sec. R.S. Received June 28, 1909.)

The following results are a continuation of the work of which summaries have already appeared in the 'Proceedings of the Royal Society.'[*]

These experiments have been carried out, with the same strain of Surra as was used before, at the Brown Institution and the Lister Institute.

A.—*Condition of the Animals living at the Date of the Completion of the Tables in the last Paper.*

Rats treated with Sodium Antimonyl Tartrate, 1 *per cent.* (*p.* 478).

No. 7 died 428 days after inoculation.
„ 32 „ 409 „
„ 35 „ 371 „

Rats treated with Sodium Antimonyl Tartrate, 5 *per cent., in Colonel Lambkin's Medium* (*p.* 482).

No. 13 died 216 days after inoculation.

Rats treated with Antimony (*metal*), 5 *per cent., in Colonel Lambkin's Medium* (*p.* 483).

No. 10 died 205 days after inoculation.
„ 17 „ 367 „
„ 25 „ 385 „
„ 27 „ 399 „
„ 29 „ 360 „

Rats treated with Lithium Antimonyl Tartrate, 0·25 *per cent.* (*p.* 485).

No. 4 died 145 days after inoculation.
„ 5 „ 229 „
„ 6 „ 257 „
„ 8 „ 241 „
„ 10 „ 209 „

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[*] B, vol. 79, 1907, pp. 500—516; and B, vol. 80, 1908, pp. 1—12 and 477—487.

Most of the above rats died from cold; none of them died from the disease, and no trypanosomes were found in their blood or organs, and inoculations made therefrom were entirely negative.

B.—*Further Experiments.*

Rats treated with Lithium Antimonyl Tartrate, 1 *per cent.*

A further series of experiments has been carried out with this substance on a large number of rats, giving four doses of 4 to 5 minims (according to weight) of a 1 per cent. solution subcutaneously, a dose being given every other day. Practically by this method every rat can be cured. They have lived for varying periods, up to 249 days, and in no case have trypanosomes been found after death in the blood or in the organs. No rat has died of the disease, and in no case thus treated has there been a recurrence. The results have therefore been more constant than those attained with sodium or potassium antimonyl tartrates. The treatment was begun on the third or fourth day after inoculation; it will be seen below that when it is left until the number of trypanosomes in the peripheral blood is very great, although they may be driven out of the blood, it does not cure: so that the time at which treatment is commenced is of considerable importance.

It has also been given intravenously in rabbits, but with far less effect than when given subcutaneously. The elimination in this case is very rapid, to which fact we attribute its comparatively feeble action.

Rats treated with Lithium Antimonyl Tartrate on the Fifth or Sixth Day of the Disease.

The blood at this period of the disease is swarming with trypanosomes, and experiments were made in order to see what effect this salt of antimony would have upon the disease at this period. If one dose of 5 minims of a 1 per cent. solution be given the rats die on the seventh day, so that little or no effect is produced. If two such doses be given, one on the fifth and one on the sixth day, the average time of death in 10 rats was 19½ days, and living trypanosomes were found in the blood at death. When four doses were given, one on each day from the fifth to the eighth, the time in three rats was lengthened to 81 to 86 days: in one of these even living trypanosomes were found in the blood after death. By comparing these results with those mentioned in the former section it will be seen that the time at which the administration of the drug is begun is of importance, as well as the number of doses. The animals stand the best chance of cure when no recurrences take place, and this is best ensured by the method described in the previous section.

Further experiments made with Rats treated with Antimony in order to find out in what Organs the Trypanosomes are latent.

Following on the experiments made on rats treated with sodium antimonyl tartrate, with the view of finding out where the trypanosomes are latent, and recorded in the last paper,* a further series of experiments has been made on rats inoculated with Surra, which is more amenable to treatment with antimony than the Nagana used in the former series, and completely treated (that is, given a curative series of doses) with lithium antimonyl tartrate: this, as stated in the paper referred to, appears to be the most active of this variety of salt.

Seven rats were treated with four doses of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate, and they were killed in succession, one on the 6th, 7th, 10th, 14th, 16th, 22nd, and 30th days after the last dose. The livers and bone-marrows were made into an emulsion with the minimum quantity of 0·89 per cent. salt solution, and 1 c.c. of the emulsions of these organs and 1 c.c. of heart's blood was injected separately into other rats. The results were entirely negative. Microscopic preparations were made of the material injected and no organisms were seen, and none of the sub-inoculations gave a positive result.

Experiments made in order to see if any Protection was afforded by Initial Treatment with Antimony.

A series of six rats was treated with four doses of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate, one dose every other day in the same manner as when given for curative purposes. They were then inoculated with Surra, one on the first day after the completion of the treatment, and one on the 2nd, 4th, 5th, 9th, and 10th days after. They all died on the 5th or 6th days after inoculation, just as untreated rats would have done, so that antimony in this very soluble form is of no protective use in rats, owing most probably to its rapid elimination.

The blood of an uninfected rat treated as above has also been used in the *in vitro* experiments recorded below.

Rats treated with Sodium Antimony Lactate and with Antimony Sodium Calcium Lactate.

Through the kindness of Messrs. von Heyden we have been enabled to make some experiments with the above compounds. The sodium antimony lactate contains 26 per cent. of antimony, and the antimony sodium calcium lactate 17 per cent., so they are both much weaker in antimony than the tartrates which we have used. By the addition of a small quantity of lactic acid we were able to get a 1 per cent. solution of both salts, and in this strength the solutions were not very irritating, but neither with rats nor with larger animals are they as effective as the tartrates or the metal.

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* 'Roy. Soc. Proc.,' B, vol. 80, p. 487.

The following table shows the results obtained with sodium antimony lactate 1 per cent.

Average duration of untreated disease 6·9 days :—

Rats of 150 to 200 grammes weight.	Number of doses, and quantity.	Recurrences.	Lived.	Remarks.
1	2 of 4 minims	0	9 days	Died from enteritis.
2	4 of 4 ,,	0	20 ,,	Died from retained fœtus.
3	4 of 4 ,,	1	37 ,,	
4	5 of 4 ,,	1	46 ,,	
5	6 of 4 ,,	2	100 ,,	(No trypanosomes found in any of these rats after death.)
6	4 of 5 ,,	0	74 ,,	
7	5 of 5 ,,	1	48 ,,	

The following table shows the results obtained with antimony sodium calcium lactate 1 per cent.

Average duration of untreated disease 6·9 days :—

Rats of 150 to 200 grammes weight.	Number of doses, and quantity.	Recurrences.	Lived.	Remarks.
1	3 of 4 minims	1	83 days	No trypanosomes found *post mortem.*
2	4 of 4 ,,	0	25 ,,	Living trypanosomes found *post mortem.*
3	5 of 4 ,,	2	68 ,,	No trypanosomes found *post mortem.*
4	6 of 4 ,,	2	45 ,,	,, ,, ,,
5	5 of 5 ,,	2	64 ,,	,, ,, ,,
6	6 of 5 ,,	2	68 ,,	,, ,, ,,
7	8 of 5 ,,	2	57 ,,	,, ,, ,,
8	4 of 7 ,,	0	131 ,,	,, ,, ,,

On dogs the effect was very much less marked than on rats, and an effective dose became inconveniently large.

The following experiments show the relatively greater time taken for these salts to act, as compared with the sodium or lithium antimonyl tartrates, which drive all the trypanosomes from the peripheral blood in about an hour after the dose.

A Surra rat was taken on the fourth day, when the trypanosomes are numerous in the blood, and 5 minims of a 1 per cent. solution of sodium antimony lactate were injected.

Blood was taken	and showed the following :
½ hour after injection...	Trypanosomes affected by the drug: are extremely active, and show a tendency to swell.
1 „ „ ...	Very few normal trypanosomes to be seen: nearly all are swollen and spherical in shape (= "battledores"). Still large numbers.
1½ hours „ ...	Much smaller number of trypanosomes to be seen: a few "battledores": a few motionless ones, and one or two normal forms.
2 „ „ ...	"Battledores" have all disappeared: one or two slowly moving normal forms seen.
2½ „ „ ...	Ditto.
3½ „ „ ...	No trypanosomes found.

A similar experiment made with a rat treated with antimony sodium calcium lactate yielded practically the same result. Further experiments made with these drugs *in vitro* will be mentioned later.

Experimen made with Antimony (Metal in state of finest Division) suspended in various Oily Media.

Since the curative results following treatment with the metal antimony* suspended in Colonel Lambkin's medium seemed promising, many trials have been made with the metal suspended in other oily media, such as olive oil, cod liver oil, lanolin, egg-yolk, etc., in order, if possible, to obviate, or at any rate reduce, the extremely irritating properties of the metal, which seriously interfere with its practical use.

In olive oil a 5 per cent. suspension was used: with one dose of 3 minims Surra rats lived for 15 days, and died with living trypanosomes in their blood. Seventeen Surra rats were given one dose of 5 minims on the fourth day of the disease, and they lived from 41 to 133 days: in these there were no recurrences, nor were trypanosomes found after death, and sub-inoculations were in every case negative. Six Surra rats were treated with the same dose in order to observe the time taken for the complete disappearance of the trypanosomes from the blood.

Blood was taken	and showed the following :
½ hour after injection...	Trypanosomes very active.
1 „ „ ...	As numerous: show evidences of swelling.
1½ hours „ ...	Still numerous: nearly all swollen: some "battledores."
2 „ „ ...	Very few forms found: all "battledores."
2½ „ „ ...	No trypanosomes seen.

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* 'Roy. Soc. Proc.,' B, vol. 80, p. 483.

Two Surra rats were taken on the fifth day, when the blood was swarming with trypanosomes, and 6 minims were given. Two and a-half hours after the rats were killed, and smears were made from the lungs, liver, spleen, kidney, bone-marrow, heart's blood, and brain. In none of the specimens could a trypanosome be found after prolonged examination.

This oil was also given to several rats upon recurrences after treatment with small doses of the lactates mentioned above: in these cases the effect was much less marked, even although the number of trypanosomes in the blood was much less than in the rats treated for the first time. This accords with our general experience that recurrences are much more difficult to deal with than the initial infection, and this applies to all the drugs we have tried.

A suspension in cod liver oil took four hours to drive the trypanosomes out of the peripheral blood.

The suspension in egg-yolk appeared to act in rats better than any other; in dogs, however, the results were variable; sometimes strikingly good, at others no better than the other mixtures: sometimes causing great irritation and sloughing, sometimes not causing any irritation at all. We have rats alive for more than 120 days after inoculation, with no recurrences, after one dose.

An experiment was made to see how long one dose took to drive the trypanosomes out of the blood. A Surra rat on the fourth day was treated with 5 minims of a 5 per cent. suspension.

Blood was taken	and showed the following:
¾ hour after injection…	Trypanosomes much affected, but not decreased. Many "battledore" forms.
1¼ hours „ …	Trypanosomes reduced in numbers: all swollen and "battledore" forms: very little movement.
2½ „ „ …	No trypanosomes found.

Experiments with Quassia.

Dr. Guillemard, of Cambridge, suggested that quassia, on account of its known poisonous effects on some of the lower forms of life, should be tested for its trypanocidal qualities. A series of experiments was therefore undertaken on rats.

Six Surra rats were treated on the third and following days of the disease with a 5 per cent. solution of the pharmacopœal extract of quassia: they were given three doses subcutaneously—5 minims on the third day, 10 minims on the fourth, and 10 minims on the fifth day. The trypanosomes were entirely unaffected, and the animals died on the sixth—seventh day.

Another series of 12 Surra rats was treated with a two hours' decoction of quassia-wood made with the minimum amount of water. Of this three doses were given—5 minims on the third day and 10 minims on the fourth and fifth days. The trypanosomes in these rats were also entirely unaffected, and the animals died on the sixth—seventh day. It was also tried intravenously in rabbits in doses of 30 minims of the decoction: no effect was produced, and the rabbits died on or about the 42nd day.

Experiments made *in vitro* correspond with these results, and will be described later.

Experiments with Arsenophenylglycin.

Professor Ehrlich kindly sent some of this substance to Dr. Bagshawe, the Director of the Sleeping Sickness Bureau, with which we have made some initial experiments upon rats. Ehrlich found that Nagana mice could be cured, in practically every case, with this substance. But the effects on larger animals, so far as we have gone, are not quite so satisfactory, and it compares in this undesirable manner very well with the antimony tartrates, with which we can cure practically every case of Surra in rats, but which do not have anything like the corresponding effects on rabbits, guinea-pigs, and dogs. It is not only in the question of practical dosage that difficulties arise: each kind of animal has a personal equation, and their reaction to a given drug is not similar. This, and the relatively larger dosage in bigger animals, present considerable practical difficulties in the treatment of trypanosomiasis.

Our experiments have given the following results. Out of eight Surra rats of 180 to 200 grammes weight which were given one dose of 25 minims of a 1 in 80 solution of arsenophenylglycin, four died on the 19th day with living trypanosomes in their blood, the recurrences having taken place on the 16th—17th day. Two were given three and five doses respectively of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate on the 17th and following days, and they lived 59 and 51 days. Of the two which are still living (95 days), one has had five doses of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate, beginning on the 17th day, and the other had one similar dose given on the day before the recurrences occurred in the other rats.

The following experiment shows the effect of this substance upon the trypanosomes in the blood, and how much longer it takes than the antimony salts to produce its effects.

A Surra rat on the fourth day of the disease was treated with 1 c.c. of a 2 per cent. solution of arsenophenylglycin (practically the same dose as given to the other rats).

Blood was taken	and showed the following:
½ hour after injection...	Trypanosomes showed slight increase of motility.
1 „ „ ...	Ditto.
2 hours „ ...	Ditto, but more marked.
3 „ „ ...	Trypanosomes not quite so active and fewer in number.
4 „ „ ...	Trypanosomes now very few in number.
4½ „ „ ...	Only one or two trypanosomes to be seen in a preparation.
5 „ „ ...	No trypanosomes seen.

In these specimens no swollen, breaking up, or "battledore" forms were seen: the trypanosomes simply disappeared.

On the Effects of the Drugs used upon the Trypanosomes in the Living Body.

In studying the therapeutic effect of the various drugs tried, including metallic antimony in a state of finest division, repeated observations of the peripheral blood were made in order to observe the effect of the drug upon the trypanosomes, and to ascertain when the trypanosomes entirely disappeared from the blood. The first stage noticed of the effect of the drug was a great increase in the motility of the trypanosomes, followed by a gradual slowing down to movements slower than normal. At this stage there is a tendency for the whole trypanosome to swell, and to become bloated in appearance. The swelling of the trypanosome continues until it becomes almost spherical in form, or oftener "battledore" shaped; the protoplasm becomes indistinct, and the flagellum appears to be attached to only one side of the periphery; the macro-nucleus is fairly distinct, but it eventually breaks up, and then the swollen mass disintegrates. The spleen at this time is full of these broken up masses of trypanosomes, and as the nuclei will still stain, a plasmodial appearance is seen in films of bits of nuclei dotted about in a granular ground. These stages can be observed after treatment with all the salts of antimony used, and are well marked after the administration of the metal, in which case, however, the stages are slower. The soluble salts, lithium and sodium antimonyl tartrates, effect the total disappearance of the trypanosomes in about one hour. Metallic antimony, when given in the various media tried (Lambkin's medium, olive oil, cod liver oil, heavy paraffin oil, egg-yolk), brings about this disappearance in from two-and-a-half to four hours, according to the medium used: the first noticeable effects being produced in about half an hour. In the case of egg-yolk and olive oil the blood is free from trypanosomes in two-and-a-half hours. This would seem to show that some portion of the metal introduced must be changed into some soluble form very rapidly; but apparently after the reaction of the tissues occurs the antimony becomes more or less shut off, and absorption must take place very slowly, as traces of the metal, apparently unaltered, have been found as late as six to seven weeks after the injection.

Sodium antimony lactate and antimony sodium calcium lactate were found to act rather more slowly than the above (see Table above), the time at which the trypanosomes had completely disappeared varying from three to four hours.

It was noticed in these experiments that trypanosomes, though obviously drug-affected when the blood was taken, remained alive on the slide outside the body for a long time after all forms had disappeared from the circulating blood.

Further details of the time taken for the various drugs to act will be found in the sections upon sodium antimony lactate, antimony oil, antimony egg-yolk, and arsenophenylglycin.

On the Action of Trypanocidal Substances in vitro.

Experiments have been carried out with a view of throwing light on the more exact nature of the changes which are produced in trypanosomes when they are brought into contact with trypanocidal substances. The general principles we have observed in these experiments have been: 1. To dissolve the drug in some fluid so that when it is added to the infected blood it will not cause osmosis to occur in the cellular elements of, or trypanosomes contained in the blood. (The various substances were dissolved in a 0·89 per cent. salt solution, isotonic with rat's blood which was used in these experiments.) 2. To use always equal volumes of the solution and of the affected blood. 3. To use blood at the time when the trypanosomes are just becoming very numerous, so as to avoid the presence of old, feebly moving forms, which are always present in the later stages of an acute infection. The method of observation has been to watch the behaviour of the trypanosomes when in contact with the various solutions of the drug under the microscope. A measured drop of blood and of the solution are mixed on a slide with care: the mixed drop is then covered with a sufficiently large cover glass, and this is sealed with vaseline.

It has been found possible in this manner to exactly determine the dilutions at which the various drugs used cease to have an instantaneously trypanocidal action; further, in higher dilutions, by carefully watching the changes taking place in the trypanosomes, it is possible to determine the dilution at which no effect is produced, and between these two points the periods of time necessary to ensure immobility and death of the trypanosomes can be ascertained. By a comparison of the results obtained a very good estimate of the probable action of any drug when given to an affected animal can be arrived at.

For instance, sodium and lithium antimonyl tartrates were found to act, in the same dilutions, in a manner fairly comparable to their antimony content, and to their action on the trypanosomes in an affected animal. Again, with atoxyl a much higher concentration of the drug was necessary—it had to be about ten times stronger—in order to obtain the same destruction pictures, results corresponding with the rapidity of the disappearance of trypanosomes from the peripheral blood of affected animals when treated with the above drugs.

In the case of the two new lactates mentioned above, their therapeutical value was accurately foretold by a preliminary study of their action *in vitro* in the manner described. In all these experiments controls have been carried out: it has been found that trypanosomes will live and retain their activity for hours when infected blood and the diluting fluid alone are mixed together.

The various changes taking place in trypanosomes on coming into contact with a dilute trypanocidal drug, commencing with their preliminary extraordinary increase of activity, and their subsequent swelling up, immobility, and disintegration, can be watched in all their different stages in this manner. These effects resemble very closely the changes which take place in the trypanosomes in the peripheral circulation of an animal treated with antimony.

The following tables show the effects produced by the different substances in their various dilutions.

Dilutions of sodium antimonyl tartrate in 0·89 per cent. salt solution mixed with Surra rat's blood, in equal parts. The control in all cases is equal parts of blood and 0·89 per cent. salt solution.

Dilutions.				Time.	Control.
1—500.	1—1000.	1—5000.	1—10,000.		
Motionless	Motionless	Few active forms	Trypanosomes active	1 min.	Very active.
„	„	Motionless	Few active forms; rest sluggish	10 mins.	„
„	„	„	All sluggish	30 „	„
„	„	„	Motionless	1 hour	„

Dilutions of lithium antimonyl tartrate in 0·89 per cent. salt solution mixed with Surra rat's blood, in equal parts.

Dilutions.					Time.	Control.
1—500.	1—1000.	1—5000.	1—10,000.	1—20,000.		
Motionless	Motionless	Some active trypanosomes	Active trypanosomes	Very active trypanosomes	1 min.	Very active.
„	„	Motionless	Some active trypanosomes	Many active trypanosomes	10 mins.	„
„	„	„	Practically no motile trypanosomes seen, only 1 or 2 in a slide. Tendency to clump	Few active trypanosomes seen. Tendency to clump	30 „	„
„	„	„	Motionless	1 or 2 active forms seen. Rest motionless	1 hour	„

In a dilution experiment with lithium antimonyl tartrate made with the blood of a Surra rat after a second recurrence, after treatment with antimony (metal) and on first recurrence with lithium antimonyl tartrate, the trypanosomes *in vitro* appeared to have a greater resistance to the dilute drug than the stock strain.

A comparison of the following table with the previous one will demonstrate this :—

Dilutions.		Time.
1—1000.	1—5000.	
A few active forms present	A number of active forms present	1 min.
Motionless	A few active forms seen	10 mins.
„	Motionless	30 „

This bears out our experience that the recurrences become less and less amenable to antimony as they increase in number.

The following table shows the action of atoxyl and lithium antimonyl tartrate compared in the above manner :—

Dilutions of atoxyl.			Time.	Dilutions of lithium antimonyl tartrate.		
1—500.	1—1000.	1—5000.		1—500.	1—5000.	1—10,000.
Trypanosomes, all active	Active	Active	1 min.	Trypanosomes, all motionless	All markedly affected	All fairly active.
Active but affected	„	„	5 mins.	Motionless: commencing disintegration	Motionless	Less active.
Less active	Sluggish, but still many active	„	15 „	Only *débris* seen	„	Some still moving; tendency to clump.
Practically motionless	Nearly all motionless; 1 or 2 active forms seen	Many moving still	2 hrs.	„	Disintegrated	Motionless; some disintegration.

Concentrated decoction of quassia in 0·89 per cent. salt solution mixed with Surra rat's blood in equal parts.

Dilutions.				Time.	Control.
1—500.	1—1000.	1—5000.	1—10,000.		
Very active	Very active	Very active	Very active	1 min.	Very active.
„	„	„	„	10 mins.	„
„	„	„	„	30 „	„
„	„	„	„	1 hour	„
Less active	Less active	Less active	Less active	2 hours	Less active.

The conditions of the dilutions and the control were precisely similar at the end of two hours. There was no swelling nor clumping.

Dilutions of arsenophenylglycin in 0·89 per cent. salt solution mixed with Surra rat's blood in equal parts.

Dilutions.					Time.	Control.
1—100.	1—500.	1—1000.	1—5000.	1—10,000.		
Very active	Very active	Very active	Very active	Very active	1 min.	Very active.
Irritated: movements rapid and convulsive	Activity increased	„	„	„	10 mins.	„
Nearly motionless	Sluggish	„	„	„	30 „	„
Motionless	„	„	„	„	1 hour	„

Experiments in vitro *performed with the Blood of a Normal Rat which had been treated with Antimony.*

Experiments were made in order to ascertain whether the blood of a rat which had been treated with antimony would show any active trypanocidal powers *in vitro.* Although in the case of an infected animal all the trypanosomes in the peripheral blood would have been destroyed in about an hour, no noticeable trypanocidal effects were shown by the blood of a treated rat in the following experiments.

A normal rat had 5 minims of a 1 per cent. solution of lithium antimonyl tartrate injected subcutaneously: its blood was taken at 15, 30, 60, and 70 minutes after the injection, and was mixed with an equal quantity of blood from a Surra rat containing many trypanosomes; the mixed bloods, taken at the times mentioned, were examined under the microscope at various intervals from 5 to 30 minutes after the mixing, and the trypanosomes were found to be entirely unaffected, so that the blood of the treated normal rat did not have any trypanocidal effect added to it by the dose of lithium antimonyl tartrate. The Surra rat, whose blood was used for this experiment, was then given 5 minims of a 1 per cent. solution of lithium antimonyl tartrate:—

Blood was taken	and showed the following:
10 minutes after injection...	Trypanosomes affected: movement very rapid.
20 „ „ ...	Many "battledores."
40 „ „ ...	Trypanosomes greatly decreased in number all "battledores."
60 „ „ ...	Blood quite free from trypanosomes.

A normal rat was given four doses subcutaneously, one every other day, of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate: 24 hours after the last dose a drop of its blood was mixed with a drop of blood from a Surra rat in which trypanosomes were plentiful. The mixture was watched under the microscope for half an hour, but no effect was produced: the blood of the treated animal behaving just as the blood of the control, an untreated rat.

A normal rat was given subcutaneously 10 minims (a lethal dose) of a 1 per cent. solution of lithium antimonyl tartrate, and its blood was mixed at half an hour, one hour, and one-and-a-half hours after the injection with an equal part of an emulsion of trypanosomes prepared from the lungs, liver, and heart's blood of a Surra rat just dead. Each of the mixtures was examined up to 30 minutes, but no effect whatever was produced on the trypanosomes. These experiments may be compared with those recorded on p. 356.

Experiments with Antimony upon Dogs.

Since the date of the last paper a large number of experiments have been made with antimony in various forms upon dogs suffering from Surra. Of the five dogs mentioned there one remains alive and well at the present date, more than a year after inoculation.

Our experiences with dogs show that they are extremely susceptible both to the disease and also to antimony: they are therefore not quite suitable animals for these experiments, although they have all lived many times the length of the untreated disease, that is 14 days. Five of the dogs were treated with small doses of sodium antimonyl tartrate in their drinking water, but the disease is so acute in dogs that this method of giving the drug, although it appeared to have some effect in postponing the reappearance of the trypanosomes in the blood, did not produce results sufficiently encouraging to warrant further experiments.

With regard to the experiments made with metallic antimony suspended in egg-yolk, the initial experiment was so encouraging as to make a further trial necessary. In this case the dog at the first relapse was given 20 minims of a 2½ per cent. suspension: there was no local reaction, which in dogs is of frequent occurrence after the administration of antimony in any form, and the trypanosomes, which were very numerous, were entirely absent from the blood in 24 hours; the dog remained quite free from them for 48 days, and gained 3 lbs. in weight, and appeared perfectly well. The recurrence was very sudden, as the dog was perfectly well up to the moment when he was seized with a series of fits which ushered in the recurrence, from which he did not recover. A rat treated at the same time as this dog with 5 minims of the same suspension is alive and well more than 100 days after this one dose.

Many of the dogs mentioned in the table below have died with fits and paralyses and other nervous symptoms, but we are not certain whether these are due to the disease or to the antimony. In certain of the dogs the treatment has appeared to alter the acute disease into a chronic one, and in one of these more chronic cases there was a considerable excess of cerebro-spinal fluid and a cellular exudation around the vessels in the brain, very similar in incidence and extent to that described and figured by one of us in rats dead from infection with *Trypanosoma gambiense.**

There is a curious uncertainty in the local effects produced in dogs by antimony, whether injected subcutaneously or intramuscularly, and they vary from time to time in the same dog; sometimes little or no effect is produced, and sometimes the suppuration and necrosis produced are sufficient to kill the animal.

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* 'Roy. Soc. Proc.,' B, vol. 79, p. 95.

We have recently given 24 injections of lithium antimonyl tartrate subcutaneously to three dogs in the greatest possible dilution: of these three places have suppurated slightly, although the conditions under which they were given were similar to those under which the 21 other doses were given. (These dogs are now living and well 53 days after inoculation, and they have had no recurrences.)

The following table gives a synopsis of the treatment, etc., of Surra dogs:—

Average Duration of Untreated Disease, 14 Days.

No.	Weight, in kilos.	Number of doses.	Quantity of dose, in minims.	Material.	Recurrences.	Remarks.
1	11	2	20	5 per cent. ant. cream	2	*Dog is alive and well* 373 *days after inoculation.*
		2	20	2 ,, sod. ant. tart.		
2	11	4	20	2 ,, ,, ,,	3	Died on 94th day: no trypanosomes found for 21 days before death. There were 41 days between the first and second recurrences. Died with fits and nervous symptoms.
		8	20	2 ,, lith. ant. tart.		
3	18¾	4	20	5 ,, sod. ant. tart. cream	6	Died on 67th day: no trypanosomes found for 22 days before death. Died with fits and nervous symptoms.
		2	20	2 ,, ,, ,,		
		1	20	5 ,, ant. cream		
		4	20	2 ,, lith. ant. tart.		
4	8	3	12	5 ,, sod. ant. tart. cream	4	Died of distemper on 63rd day. No trypanosomes found for 7 days before death.
		1	20	2 ,, ,, ,,		
		7	12	2 ,, lith. ,,		
5	6¾	3	10	5 ,, sod. ,, cream	3	Died of pneumonia on the 53rd day. No trypanosomes found for 11 days before death.
		2	20	1 ,, lith. ,,		
		3	10	2 ,, ,, ,,		
6	6¼	5	10	2 ,, ,, ,,	1	Died from abscess on the 40th day. No trypanosomes found for 17 days before death.
		4	20	2 ,, ,, ,,		
7	7¼	5	10	2 ,, ,, ,,	3	Died from abscess on the 61st day. No trypanosomes in blood for 10 days before death.
		7	20	2 ,, ,, ,,		
8	14½	4	20	2 ,, ,, ,,	2	Died on 55th day with fits and nervous symptoms. Trypanosomes in blood. Antimony given in water also.
		2	12	5 ,, ant. oil		
9	12¾	2	15	2 ,, lith. ant. tart.	2	Died on 77th day from abscess. No trypanosomes seen for 16 days before death. Antimony given in water also.
		2	15	5 ,, ant. oil		
		1	20	5 ,, ,,		
10	13¼	2	12	5 ,, ,,	3	Died on 63rd day with nervous symptoms and paralysis. No trypanosomes found. Antimony given in water also.
		1	20	2 ,, lith. ant. tart.		
		2	15	2 ,, ,, ,,		
11	8½	3	20	1 ,, ant. sod. lact.	4	Died on the 66th day with nervous symptoms. No trypanosomes found after death. Antimony given in water also.
		1	15	2 ,, lith. ant. tart.		
		1	10	5 ,, ant. oil		
		1	15	5 ,, ,,		
		1	5	2½ ,, ,,		
		2	15	2½ ,, ,,		

Average Duration of Untreated Disease, 14 days.

No.	Weight, in kilos.	Number of doses.	Quantity of dose, in minims.	Material.	Recurrences.	Remarks.
12	10	1	20	1 per cent. ant. oil	3	Died on the 65th day with living trypanosomes in blood. Antimony given in water also.
		2	10	5 ,, ,,		
		2	15	5 ,, ,,		
		4	15	2 ,, lith. ant. tart.		
13	11½	1	20	2 ,, ,, ,,	1	Died on the 60th day with nervous symptoms. No trypanosomes seen for 29 days before death.
		1	15	2½ ,, ant. oil		
		2	15	5 ,, ,,		
		1	20	5 ,, ,,		
14	10½	2	15	5 ,, ,,	2	Died on the 52nd day from abscess. No trypanosomes found after death.
		1	20	5 ,, ,,		
		1	15	2 ,, lith. ant. tart.		
		2	20	2 ,, ,, ,,		
15	13¾	1	15	5 ,, ant. oil	5	Died on the 50th day with living trypanosomes in blood.
		2	20	5 ,, ,,		
		3	20	2 ,, lith. ant. tart.		
16	9¾	1	15	2 ,, ,, ,,	0	Died on the 64th day from abscess. No trypanosomes found after death and no recurrences.
		2	15	5 ,, ant. oil		
		1	10	2½ ,, egg ant.		
17	9¼	2	15	5 ,, ant. oil	0	Died on the 48th day from pneumonia. No trypanosomes found after death.
		1	15	2 ,, lith. ant. tart.		
18	9¼	1	15	2 ,, ,, ,,	1	Died on 50th day, possibly from ant. sod. lactate. No trypanosomes found after death.
		2	15	5 ,, ant. oil		
		1	20	1 ,, ant. sod. lact.		
19	10¼	1	20	2 ,, lith. ant. tart.	3	Died on 74th day with nervous symptoms. There were 48 days between the first and second recurrences.
		1	15	5 ,, ,, ,,		
		1	10	5 ,, ,, ,,		
		1	20	5 ,, ant. cream		
		1	20	2½ ,, egg ant.		
		1	10	2½ ,, ,,		
20	7¼	3	15	2¼ ,, ,,	—	Trypanosomes practically never out of blood. Died on the 37th day, paralysed.
		1	15	5 ,, ,,		

21	13½	2	10	2½ per cent. egg ant.	—	Trypanosomes practically never out of blood. Died on the 44th day with fits and nervous symptoms.
		3	20	5 " "		
		1	15	2 " lith. ant. tart.		
22	8½	3	15	2½ " egg ant.	—	Trypanosomes practically never out of blood. Died on the 55th day with fits and nervous symptoms.
		3	15	2 " lith. ant. tart.		
23	13¼	1	15	5 " egg ant.	3	Died on the 55th day with fits and nervous symptoms.
		2	20	5 " "		
		2	15	5 " lith. ant. tart.		
		4	5	5 " " "		
24	12¼	1	15	5 " egg ant.	2	Died on the 64th day with living trypanosomes in the blood.
		1	20	5 " "		
		1	15	5 " lith. ant. tart.		
		1	10	5 " " "		
		5	5	5 " " "		
25	9¾	1	10	5 " " "	2	Died on the 56th day with nervous symptoms.
		1	20	5 " egg ant.		
		5	5	5 " lith. ant. tart.		
26	8	1	10	5 " " "	2	Died on the 47th day. Trypanosomes found in the cerebro-spinal fluid.
		1	20	5 " egg ant.		
		5	5	5 " lith. ant. tart.		

Observations on the Urine in Chronic Disease of the Pancreas.

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(Communicated by Sir Victor Horsley, F.R.S. Received April 5,—Read May 20, 1909.)

Modern researches on the physiology of the pancreas have shown that it plays a much more important part in digestion than had formerly been supposed, and also indicated that it exerts a very considerable influence on the internal metabolism of the body. The present investigations were commenced early in 1901, at the suggestion of Mr. Mayo Robson, with the object of throwing further light on the nature of these metabolic processes and discovering, if possible, some more reliable means of diagnosing diseases of the pancreas than is usually afforded by the clinical signs.

The condition of the blood in patients suffering from diseases of the pancreas was first investigated, and subsequently attention was devoted to the urine. The clinical bearing of the results of these observations has been dealt with in my Arris and Gale Lecture, and Mr. Robson's Hunterian Lectures, delivered at the Royal College of Surgeons in 1904,* and also in a paper I read before the Royal Medical and Chirurgical Society in 1906.†

In the course of a series of experiments designed to discover whether glycerine, the soluble product of the disseminated fat necrosis met with in pancreatitis, or some derivative of it, was excreted by the kidneys, I found that if the urine of a person suffering from an inflammatory affection of the pancreas were boiled with hydrochloric acid, the excess of acid neutralised with lead carbonate, and the freed glycuronic acid precipitated out of the acid solution with tri-basic lead acetate, treatment of the filtrate with phenylhydrazin yielded a crystalline product which appeared to vary in amount with the stage and intensity of the disease, while normal urines and specimens from patients suffering from diseases in which there was no reason to think that the pancreas was involved gave no reaction. In this communication I propose to describe a series of experiments I have conducted into the nature of this product, and also the results of animal experiments designed to discover whether the reaction depended upon changes in the pancreas itself or was due to alterations in the other tissues of the body brought about by a disturbance of its metabolic functions.

For the purpose of the former investigations 4 litres of urine from each of eight patients, under the care of Mr. Mayo Robson, were collected and separately examined, but as the methods employed and the results obtained were similar in all it will not be necessary to deal with them individually. The diagnosis of "pancreatitis" in these cases was based upon the clinical symptoms, analyses of the fæces, and the condition of the pancreas found at operation, while in one instance it was confirmed by histological examination of the gland after death. In five the pancreatitis was associated with, and was probably dependent upon, the presence of gall-stones in the common bile duct; two had had symptoms of "indigestion" for several years, which in one was believed to have originated in an attack of typhoid fever, and in the other had come on insidiously, but was associated with evidence pointing to excessive putrefactive changes in the contents of the intestine and an abnormal intestinal flora. In one there was sub-acute pancreatitis with disseminated fat necrosis, and an impacted gall-stone in the ampulla of Vater; *post-mortem* examination in this case showed an abscess in the tail of the pancreas and staining of the walls of the duct of Wirsung, with bile for a distance of about 3 inches from its junction with the common bile duct. Sections of the gland on microscopical examination showed a considerable over-growth of the interlobular connective tissue with some small round-celled inflammatory infiltration and vacuolisation of the acinar cells, which stained feebly.

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* 'Lancet,' March 19, 26, April 2, 1904.

† 'Roy. Med. Chi. Soc. Trans.,' 1906.

The urine from these patients, which had been preserved with chloroform, was filtered to remove suspended matter, and to the 4 litres were added 400 c.c. of hydrochloric acid (sp. gr. 1·16). The mixture was placed in a flask with a funnel in the mouth to act as a condenser, and the flask heated on a sand-bath until the contents boiled. After being kept gently boiling for 10 minutes the flask was cooled in running water, the urine made up to its original volume with distilled water, and the excess of acid neutralised by slowly adding 1600 grammes of lead carbonate. On the completion of the reaction it was filtered through a moist filter-paper, and the acid filtrate shaken with 1600 grammes of tri-basic lead acetate. The filtrate from this was made alkaline with ammonia, and the resulting precipitate washed with distilled water until the washings no longer acted upon red litmus paper. The precipitate was then suspended in 200 c.c. of distilled water, made faintly acid with hydrochloric acid, treated with a stream of sulphuretted hydrogen, and the precipitated lead sulphide removed by filtration. The clear filtrate was gently warmed on a water-bath and, when free from sulphuretted hydrogen, was again well shaken with 30 grammes of tri-basic lead acetate, filtered, and the filtrate made alkaline with ammonia. The precipitate that formed, after being washed with distilled water until the washings were neutral to litmus, was suspended in 100 c.c. of distilled water, treated with sulphuretted hydrogen, filtered, and the filtrate gently warmed to expel the excess of gas. It was then cooled and again filtered. The resulting clear solution was then examined as follows:—

A. The fluid gave all the usual carbohydrate reactions: Molisch's test was positive; heating with concentrated sulphuric acid caused it to quickly blacken; Moore's test gave a brown coloration; Tollen's ammoniacal silver nitrate solution showed a black precipitate at room temperature in a few minutes; alkaline solutions of copper sulphate and bismuth were readily reduced, but a solution of copper acetate in acetic acid (Barford's test) was not reduced, showing that the carbohydrate was not dextrose. The presence of free aldehyde was excluded by the absence of any reaction with a solution of rosaniline decolorised by sulphur dioxide.

B. Examined with the polariscope the fluid was found to be optically inactive.

C. Fifty cubic centimetres of the solution were mixed with 2 grammes of phenylhydrazin hydrochlorate and 6 grammes of sodium acetate, and heated in a water-bath at 100° C. for two hours. On cooling, a dense flocculent light yellow precipitate appeared. Microscopically, this was found to consist of long, flexible, hair-like crystals, which, on being irrigated with 33-per-cent. sulphuric acid, disappeared within a few seconds of the acid reaching them, suggesting that they were either a pentosazone or maltosazone.* The precipitate was filtered off, well washed with cold distilled water, and recrystallised from hot 10-per-cent. alcohol three times. It was then dried in a hot-water oven, cooled in a desiccator, and examined.

(1) The crystals were found to be soluble in water at 60° to 70° C., like a pentosazone.†

(2) A melting-point of 178° to 180° C. was obtained with seven of the specimens; one, that obtained from the patient with sub-acute pancreatitis, softened at 160° C., but did not completely melt until the temperature reached 178° to 180° C.

(3) Estimation of the nitrogen-content by Dumas's method gave 17·02 per cent., 17·01 per cent., 16·62 per cent., 17·00 per cent., 16·40 per cent., 16·86 per cent., 17·11 per cent., 17·08 per cent. of nitrogen, readings which fall within the limits of experimental error of the calculated 17·07 per cent. of nitrogen for pentosazone.

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* 'Roy. Med. Chi. Soc. Trans.,' vol. 88, p. 265.

† Neubauer and Vogel, 'Analyse des Harns,' 1898, p. 88.

D. The aniline acetate test, when applied to the original solution,* gave a uniform bright red colour. The phloroglucin test was also positive, showing a bright red colour at the boiling-point and forming a dark purple precipitate after boiling for about one minute. Spectroscopical examination of a solution of this precipitate in 93-per-cent. alcohol revealed an absorption band in the green, to the right of D. Tollen's orcin test, and Bial's reagent† gave a dull red-brown colour and formed a precipitate, but no green-tint was observed. An extract of this made with four parts of amyl alcohol and one part of ethyl alcohol showed, however, a dark band between C and D when it was examined with the spectroscope. The solution showed no gas formation or diminution of its reducing powers after being incubated with brewer's yeast for 24 hours at 37° C.

These results indicated that the fluid prepared in the manner described from the urine of patients having the clinical signs and symptoms of pancreatitis contained an unfermentable sugar giving the reactions of a pentose. A careful examination of the urine in these cases, and also in over 500 others that have given a positive "pancreatic" reaction, failed to reveal any free sugar to which the reaction might be due, and it was therefore evident that the pentose was not free as such in the urine, but was derived from some antecedent substance in the course of the reaction. Numerous attempts have been made to isolate this and determine its nature, but so far without success. It does not appear to be precipitated by alcohol, ether, ammonium sulphate, magnesium sulphate, lead acetate, mercuric chloride, or calcium chloride. Treatment of the urine with benzoyl chloride and sodium hydrate has also given negative results.

Beside the case already mentioned, in which a detailed examination of the pancreas was made, it has been possible to investigate the condition of the gland after death in 26 others in which the urine had been examined during life. In 9, where no reaction had been obtained, it appeared to be normal both macroscopically and microscopically. In 13 there was evidence of chronic inflammation: these had all given a positive reaction. Four proved to be cases of cancer of the pancreas, 2 of these had given a positive reaction and 2 a negative result. Of the 13 that showed chronic pancreatitis, 12 were of the typical interlobular type, and 1 was in an early stage of inflammation with no marked increase of fibrous tissue, but an interlobular and intercellular infiltration of small round cells and granular changes in the acinar cells.

The occurrence of this reaction in the urine of patients who presented the clinical symptoms of pancreatitis, and its absence in those whose pancreas was not apparently diseased, together with the *post-mortem* evidence I had been able to obtain, suggested that it was due either to some degenerative change in the tissues of the pancreas or to a disturbance of metabolism set up by disease of the gland. To decide this question, and also to obtain confirmation of the dependence of the reaction upon disease of the pancreas, I arranged a series of experiments upon animals, the surgical part of which was kindly carried out for me by Mr. H. C. G. Semon, in the Pathological Department of the University of Freiburg. A detailed account of these experiments will be given subsequently, and I shall now only deal with those points that bear upon the questions at issue.

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* Mullikin, 'Identification of Pure Organic Compounds,' 1904, p. 33.

† 'Deutsch. Med. Woch.,' 1902 and 1903.

The urine from the dogs employed for the experiments was drawn by catheter both before and after the operations. Each specimen was shaken with a few drops of chloroform, sealed in a glass vessel, marked with a distinguishing number and letter for subsequent reference, and despatched to me in London.

The normal urine, taken before operation, gave no reaction in any of the dogs examined.

(I) A sub-acute pancreatitis was set up in the first dog by injecting a very small quantity of turpentine, less than 1 c.c., into the pancreatic duct. A specimen of the urine withdrawn 16 hours after the operation gave an exceedingly well-marked reaction, the whole bulk of the fluid being filled with a light yellow flocculent precipitate, which on microscopical examination was found to consist of long fine crystals that dissolved in 33-per-cent. sulphuric acid in 5 to 10 seconds. Filtered off, and purified by re-crystallisation from 10-per-cent. alcohol, it was found to melt at 178° to 180° C. A second specimen taken 24 hours after the operation gave a similar, but less marked, reaction.

Three days later a much larger dose of turpentine (1·5 c.c.) was injected, but by mistake this was introduced into the common bile duct instead of the pancreatic duct. The urine withdrawn six hours after this operation contained a large amount of urobilin, but gave *no* "pancreatic" reaction. Two other samples taken at the end of 18 and 24 hours respectively after the operation also yielded no osazone crystals.

A small piece of the pancreas was excised at the time of the second operation. Microscopical examination of this showed no small celled infiltration or over-growth of connective tissue, but the nuclei of the acinar cells were indistinct and the protoplasm was highly vacuolated. When the animal was killed 48 hours later examination of the pancreas revealed no pathological changes.

(II) In a second dog chronic pancreatitis was induced by passing a silk thread from the duodenum along the duct of Wirsung and leaving the loose end hanging free in the intestine.* The urine withdrawn three days after the operation yielded crowds of long fine yellow crystals, soluble in 33-per-cent. sulphuric acid in 5 to 10 seconds, and which melted at 178° to 180° C. A second specimen taken one week after the operation showed many typical crystals. A third sample obtained two weeks after the operation gave some crystals, but the reaction was not as well marked as that given by the preceding specimens. A fourth sample obtained one week later still gave a fairly well-marked reaction.

At the end of the next week the pancreas and part of the duodenum were removed and the cut ends of the intestine united by a Murphy button. Examination of the excised portions showed that the thread was still in position in the pancreatic duct and hanging into the duodenum. The whole pancreas, but more particularly the head of the gland, was thickened and felt heavier than normal. Microscopical examination of sections cut from various parts showed a marked over-growth of the interlobular connective tissue, especially in the neighbourhood of the duct of Wirsung and its larger tributaries. The epithelium of the duct was detached and in places lay loose in the lumen. The periphery of the gland was not so markedly affected, although here, too, there appeared to be some increase of fibrous tissue and a few round cells were seen in and around the ducts.

Immediately after the second operation the bladder was emptied by catheter. A specimen of urine withdrawn 15 hours later was found to contain 1·8 per cent. of reducing sugar, as estimated by Bang's method; 1·6 per cent. of fermentable sugar, estimated by Lohenstein's saccharometer, and the polariscope showed 1·2 per cent. of dextro-rotatory sugar. Treatment with phenylhydrazin gave a dense precipitate of coarse greenish-yellow crystals that were insoluble in 33-per-cent. sulphuric acid in five minutes, and on re-crystallisation from 70-per-cent. alcohol melted at 204° to 205° C., thus corresponding to dextrosazone. Forty-five cubic centimetres of the filtered urine were boiled with 3 c.c. of hydrochloric acid for 10 minutes, the excess of acid neutralised with lead carbonate, and the glycuronic acid removed by shaking with tri-basic lead acetate. The lead in the filtrate was then removed by treatment with a stream of sulphuretted hydrogen and subsequent filtration. After being heated to drive off the excess of sulphuretted hydrogen, the filtrate was cooled and mixed with half its bulk of distilled water. Yeast was then added, and the mixture incubated at

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* *Cf.* Carnot, 'Gilbert et Thoinot, Traite de Médicine et de Thérapeutique,' 1908, fasc. 20, p. 238.

37° C. for 18 hours, when, as it was found that a control specimen no longer gave a reaction for sugar, it was cooled, filtered, and the filtrate treated with phenylhydrazin. Examination 24 hours later showed no crystalline deposit, either to the naked eye or microscopically. The animal was found dead three days after the operation, and *post mortem* no trace of pancreatic tissue could be discovered.

(III) The pancreas of a third dog was extirpated on September 9. Two days later it died from gangrene of the duodenum. A specimen of urine was, however, obtained before death, and 18 hours after the operation. This was found to contain 3·9 per cent. of reducing sugar (Bang), and 3·8 per cent. on fermentation (Lohenstein). Examination with the polariscope gave 3·2 per cent. of dextro-rotatory sugar. The osazone crystals melted at 204°—205° C., and were insoluble in 33-per-cent. sulphuric acid in five minutes. A specimen of the urine examined by the same method as that just described gave no "pancreatic" reaction after the fermentable sugar had been removed, there being no crystalline deposit on macroscopical or microscopical examination of a preparation left undisturbed for 24 hours.

The results of the examination of the samples from Dog I and of the specimens obtained from Dog II after the first operation agree with and confirm my experience in the human subject. They show that while normal urine gives no "pancreatic" reaction, specimens from animals in which pancreatitis has been set up, either by a chemical irritant, such as turpentine, or bacterial infection and partial blocking of the pancreatic duct, give a characteristic reaction. The accidental injection of turpentine into the common bile duct of Dog I served to prove that the reaction first obtained was not due to the turpentine itself or to the manipulation of the organs in the course of the operation, for, although considerably larger than the first dose which was injected into the pancreatic duct, it gave rise to no urinary "pancreatic" reaction. The pathological changes found in the pancreas of Dog II, when it was removed at operation, agree with those described by Carnot as being present in similar experiments performed by him, and correspond to the chronic interlobular pancreatitis met with in man as the result of an infection of the pancreatic ducts.

The results of the examination of the urines of Dogs II and III, after total extirpation of the pancreas, tend to show that the changes which give rise to the "pancreatic" reaction in the urine are dependent upon the presence of the pancreas, and are probably to be referred to changes in the gland itself and not to disturbances of metabolism in other tissues brought about by interference with or perversion of its functions, for Dog III gave no reaction after the operation, and in Dog II the reaction which had been obtained on four occasions during the preceding three weeks disappeared after removal of the pancreas.

It may be objected that the modification of the procedure made necessary by the presence of the fermentable sugar interfered with the reliability test, but that this is not the case has been shown by the results obtained on examining the urines from several patients suffering from glycosuria associated with disease of the pancreas by the same method. One in particular demonstrated this very clearly, and also showed how an exceedingly well-marked reaction may diminish in intensity as destruction of the pancreas progresses, and finally disappears when advanced glycosuria has been established. The patient was first seen in December, 1906; there was then an abdominal tumour which was suspected to be pancreatic, but an examination of the urine gave no "pancreatic" reaction, and there was also at that time no sugar. An exploratory examination was performed by Mr. Mayo Robson, and a growth was found in the first part of the duodenum, but quite free from the pancreas. On January 18, a second specimen of urine was examined and found to be free from sugar, but it gave a well-marked "pancreatic" reaction, suggesting that the pancreas was then involved in the disease. At the request of the patient's friends the abdomen was re-opened a few days later and it was then found that the growth had invaded the pancreas as had been suspected. In the early part of May, 1906, examination of the urine showed 5·25 per cent. of sugar and a modified "pancreatic" reaction gave many fine crystals soluble in 33-per-cent. sulphuric acid in 5 to 10 seconds. A month later the sugar had increased to 7 per cent., and a much less marked "pancreatic" reaction was obtained. In July the urine contained 7·25 per cent. of sugar and the "pancreatic" reaction gave only a few crystals. In August, 7·5 per cent. of sugar was present, and no crystals were found on carrying out the modified "pancreatic" test. In October the urine contained 9·5 per cent. of sugar and the "pancreatic" reaction was negative. The patient died on November 5.

The indications afforded by the experimental, pathological, and clinical evidence so far obtained all point to the so-called "pancreatic" reaction in the urine being due to active degenerative changes in the pancreas, and, so far as I have been able to determine, to these alone. The fact that the sugar giving rise to the reaction is apparently a pentose suggests that it is probably contained in a derivative of the pancreas nucleo-protein which passes into the blood as a result of the degeneration of the gland cells. In view of the constant presence of a pentose in the nuclei of the cells of other organs and tissues it might be thought that if this were true, degeneration in these would also furnish a pentose-yielding substance which might pass into the urine, but this does not appear to be the case. The reasons why the pancreas is probably more liable to yield a pentose complex that can be split up and so recognised in the urine are two: first, according to Gründ,* the percentage of pentose in the dry weight of the pancreas is nearly five times as great as in any other organ of the body (pancreas 2·48 per cent., liver 0·56 per cent., thymus 0·56 per cent., kidney 0·49 per cent., muscle 0·11 per cent.); second, the pentose contained in the nucleo-protein of the pancreas and thymus is said to be more loosely combined and more readily set free than the corresponding sugar in other tissues.† With regard to the first point, however, the relative bulks of the organs have to be taken into account, and it is conceivable that if the whole of an organ, such as the liver, were simultaneously involved in some degenerative change, it might yield as much or more pentose-containing substance as the pancreas under similar conditions.

Only a small part of the field of research opened up by these investigations has as yet been touched upon. It is hoped that by further experiment it may be possible to isolate the mother-substance giving rise to the pentose obtained from the urine in cases of pancreatitis, and also that a fresh series of animal experiments may furnish information as to the chemical changes in the body associated with diseases of the pancreas that precede and lead up to the disturbances of internal metabolism that give rise to diabetes.

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* Hoppe-Seyler's ' Zeit. f. hysiol. Chem.,' vol. 35, p. 111.

† Blumenthal, "Dis. of Metabolism," v. Noorden's 'Clin. Med.,' 1906, p. 262.

A Method of Estimating the Total Volume of Blood contained in the Living Body.

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(Communicated by Prof. Sherrington, F.R.S. Received May 7,—Read May 20, 1909.)

(From the Liverpool School of Tropical Medicine.)

The determination during life of the total volume of blood contained in the living body is usually effected by Haldane's method.* This consists in first estimating the percentage hæmoglobin content of the blood and then determining the total hæmoglobin content of the circulating fluid, the latter being effected by administering a known volume of carbon monoxide and observing the extent to which the hæmoglobin of the red cells is in combination with this gas. Copeman and Sherrington† determined the volume of the blood in the living body by injecting a measured volume of 0·75-per-cent. solution of sodium chloride (sp. gr. 1·0046) and observing the resulting fall in specific gravity of the blood.

Recently, while making an investigation upon hæmoglobinæmia, we found it necessary to make estimations of the total amount of blood in the living body. This we carried out by: (1) making a hæmocrit estimation of the relative proportions, by volume, of red cells and plasma; (2) injecting a known quantity of dissolved hæmoglobin into the blood stream and determining the degree of the resulting hæmoglobinæmia, from which the amount of plasma present can be calculated. The method employed thus consists of two procedures, namely the estimation: (1) of the percentage (by volume) of plasma contained in the blood (A); and (2) of the total volume of plasma contained in the body (B). The total volume of the blood is $\frac{B}{A} \times 100$.

1. To carry out the first procedure 0·20 c.c. of a 1-per-cent. solution of potassium oxalate was placed in a glass capsule and about 1 c.c. of the blood of the subject of examination, removed from a vein, added. The volume of the mixture was then carefully measured, a hæmocrit determination made, and the percentage of plasma in the undiluted blood calculated therefrom.

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* "The Mass and Oxygen Capacity of the Blood in Man," 'Journ. of Physiol.,' 1899—1900, vol. 25, p. 331.

† "Method for Determining the Quantity of Blood in a Living Animal," 'Journ. of Physiol.,' 1890, vol. 11, p. 8; also "The Specific Gravity of the Blood," *ibid.*, 1893, p. 71.

An example will make this clear. In an experiment upon a rabbit the volume of the mixture was 1·12 c.c., and the proportion of plasma in the mixture determined by the hæmocrit 85·3 per cent. The proportion, by volume, of plasma in the undiluted blood was therefore

$$\left(\frac{85\cdot3}{100}\times1\cdot12-0\cdot20\right)\frac{100}{92}=82\cdot1 \text{ per cent.}$$

2. To carry out the second procedure a solution of hæmoglobin was prepared, usually by laking the red blood cells of the animal whose blood volume was to be determined, though in some experiments the red blood cells of another animal of the same species were employed instead of the animal's own red cells. To this end the required amount of blood was taken from a vein, usually by means of a cannula or hollow needle, and added to a sufficient quantity of a 1-per-cent. solution of potassium oxalate to prevent clotting. The mixture was then centrifugalised, the supernatant plasma completely removed, and to the red cells distilled water added in amount just sufficient to produce laking. When this had occurred, solid sodium chloride was added in the amount required to produce a 0·85-per-cent. solution of this salt. The red cell constituents which separated out on the addition of sodium chloride, forming a reddish white precipitate, were then separated by centrifugalisation and a dark red solution obtained. The strength of this solution was determined by matching a portion of it, suitably diluted, with a solution containing a known percentage by volume of red blood cells, the solution being prepared by adding a measured amount of oxalated blood, previously submitted to a hæmocrit determination, to distilled water. The matching was sometimes carried out in a Zeiss comparison spectroscope, but more frequently we employed the simpler plan of estimating the percentage of hæmoglobin in the diluted solution by means of v. Fleischl's hæmoglobinometer, the scale of which had been previously standardised by means of solutions of hæmoglobin representing known percentages by volume of red blood cells. In this way the strength of the hæmoglobin solution employed, which usually represented the amount contained in 25 to 40 per cent. of its volume of red cells, is determined. A measured volume of this solution, corresponding to a known volume of red cells, was then injected into a vein of the animal whose blood volume was to be ascertained. As soon as the injection was completed a sample of blood was taken from a vein on the opposite side of the body, added to a known quantity of a 1-per-cent. solution of potassium oxalate, and the volume of the mixture measured. The mixture was then centrifugalised and the percentage of dissolved hæmoglobin determined as above described.

If the plasma, obtained before injection, contained a small percentage of dissolved hæmoglobin, as usually happens in the human subject, this was subtracted from that present after injection of hæmoglobin solution.

When the total amount of hæmoglobin injected (C) has been determined, and also the percentage of hæmoglobin contained in the blood plasma after injection (D), the total amount of blood plasma after injection (E) is calculated from the formula

$$E = \frac{C}{D} \times 100.$$

From this the amount of fluid injected was subtracted, and to it should be added the amount of plasma withdrawn, if the animal had been bled before injection. By way of illustration the continuation of the experiment upon a rabbit, already referred to in (1), is shown below, the amount of hæmoglobin (C) being expressed as before in terms of red blood cells in the moist condition, the volume of red cells being given instead of their weight in order to avoid calculation of the latter, and the percentage of hæmoglobin (D) being similarly expressed in equivalent volume of red cells.

Amount of dissolved hæmoglobin injected into blood stream. C.	Percentage of hæmoglobin dissolved in blood plasma after injection. D.	Total volume of blood plasma. $\frac{C}{D} \times 100$.	Total volume of blood [hæmocrit determination gave 82·1 per cent. by volume of plasma in undiluted blood].
0·74 c.c. dissolved in 0·85-per-cent. NaCl solution, the total volume of fluid being 4·0 c.c.	0·73 per cent.	101 c.c. after injection ∴ 97 c.c. before injection	$\frac{97 \times 100{\cdot}0}{82{\cdot}1} = 118$ c.c.

In no case could any change in the general condition of the rabbits be observed after the injection of dissolved hæmoglobin. In man the injection of dissolved hæmoglobin, obtained from the subject's own red cells, in amount sufficient to cause the blood plasma to contain as much hæmoglobin as was present in 1 per cent. of its volume of red blood cells, also produced no alteration of the general condition. Aseptic precautions were employed throughout these procedures.

The amount of hæmoglobin can be varied within wide limits. If, however, the blood plasma contains less dissolved hæmoglobin than would be represented by 1 per cent. of its volume of red blood cells, the natural colour of the normal plasma may interfere with the hæmoglobinometer determination. This is likely to occur if the blood plasma before injection is unusually dark, as sometimes occurs in pathological conditions attended with red cell destruction. In such cases, and also when the degree of hæmoglobinæmia was slight, we employed a Zeiss comparison spectroscope, provided with a cell of variable height, the estimation being made by matching the oxyhæmoglobin bands of the plasma with those of a solution of hæmoglobin of known concentration.

The accuracy of this method of estimating the total volume of the blood depends upon the precision with which : (1) the hæmoglobinometer readings (or the comparison spectroscope determinations) are made ; (2) the condition of the plasma immediately after injection is ascertained.

In our observations the variation of successive hæmoglobinometer readings of the same solution of hæmoglobin did not exceed ± 2 per cent. of the mean reading.

The mode of ascertaining the degree of hæmoglobinæmia immediately after injection is a matter of importance. If only a small percentage of hæmoglobin, representing less than 1 per cent. by volume of red cells, is present, no correction is ordinarily required when the plasma is obtained within three minutes of the injection, which generally occupies about one minute. If, however, a sample of blood cannot be obtained so soon after injection, or again if a much higher degree of hæmoglobinæmia has been produced, then an amount of dissolved hæmoglobin sufficiently large to affect the determination of the blood volume may have disappeared before the sample is obtained. In such cases, two or three determinations of the degree of hæmoglobinæmia require to be made at short intervals as soon as possible after injection. From these the percentage of dissolved hæmoglobin present in the plasma immediately after injection can be calculated. In a series of observations upon the rate of disappearance of dissolved hæmoglobin from the blood plasma in the living body, which will shortly be published,* the mode in which this calculation may be made will appear.

The advantage of the above method lies in the ease of its application, and the circumstance that the injection of hæmoglobin is not attended with any recognisable alteration of the general condition.

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* 'Annals of Tropical Medicine and Parasitology,' 1909, vol. 3, p. 1.

Preliminary Note on Trypanosoma eberthi (*Kent*) (= Spirochæta eberthi, *Lühe*) *and some other Parasitic Forms from the Intestine of the Fowl.*

By C. H. Martin, B.A., Demonstrator of Zoology in Glasgow University, and Muriel Robertson, M.A., Carnegie Research Fellow, Assistant to the Professor of Protozoology in the University of London.

(Communicated by Prof. J. Graham Kerr, F.R.S. Received June 18,—Read June 24, 1909.)

[Plate 8.]

In the eleventh volume of the 'Zeitschrift für Wissenschaftliche Zoologie,' published in 1862 (p. 98), Eberth described, in a remarkable paper, "ein kleines Infusorium" which he had found abundantly in the cæca of various birds (fowl, partridge, duck, goose); he described it as a flattened crescentic form measuring 0·012 to 0·014 mm. long by 0·006 to 0·008 mm. wide, with a wide and a narrow extremity, the latter of which is drawn out into a slight point. He distinguished the true body of the animal, in which he sometimes thought he saw a nucleus, from the conspicuous "häutige Saum," the movements and appearance of which he described and figured very clearly. He considers that this form is related to the form seen by Leydig in the gut of Piscicola, Pontobdella, and Ixodes, which Leydig had believed came from the blood of fish, and those described in the blood of various fish and of the frog. Eberth, however, remarked that he could not find his parasite in the blood of the infected birds, though he found two other flagellates, which he does not further describe, in the intestine.

Leuckart, in his first edition (1863) of 'Die Parasiten des Menschen' (vol. 1, p. 140), placed the animal described by Eberth in a new genus, *Sœnolophus*; in his second edition, however, under Stein's influence (*vide infra*), he came to the conclusion that this animal "Vermutlich gleichfalls nichts Anderes als eine *Trichomonas* ist, bei der die Anwesenheit des Geisselapparates übersehen wurde" (2nd Edition, p. 312).

Stein (1878) was inclined to consider the parasite as a Trichomonas of which the anterior flagella had been overlooked. ('Der Organismus der Infusionsthiere,' Abtheilung. III, 1. Hälfte, p. 79. "Sollte nun das vordere End, wie ich vermuthe, noch mit zwei Geisseln versehen sein, so würde dieser Parasit, aus dem Leuckart sogleich eine neue *Sœnolophus* gemacht hat, unbedingt zur Gatt. Trichomonas gehören.")

The next reference to this form that we have found is to be met with in

Saville Kent's 'Monograph of the Infusoria' (1881), where it is named *Trypanosoma eberthi.* Saville Kent has apparently overlooked Leuckart and Stein's references to this form, and his theory as to its possible connection with the trypanosoma of the frog need not be considered here.

Bütschli (1889), in his account of the Protozoa, gave Eberth's figures of this form and placed it in the genus Trypanosoma with the true blood parasites.

Doflein, in 1901, gave Eberth's description and figures of this form, which he seems inclined to connect with some of the flagellate parasites seen in "Geflügel-Diphtherie." The evidence on which he does this seems rather scanty and he himself remarks (p. 60): "Mir scheint aus dem Studium der Literatur hervorzugehen, dass zwei oder mehr verschiedene Arten in den Angaben mit einander vermengt worden sind. Es wäre sehr verdienstlich in dieser interessanten Frage Klarheit zu schaffen."

Laveran and Mesnil (1904), in their 'Trypanosomes et Trypanosomiases,' state, on p. 354, "Il paraît bien certain que le *Trypanosoma eberthi,* Kent, vu par Eberth dans le tube digestif de la poule, n'est pas un Trypanosome, c'est sans doute un Trichomonas, comme Stein, puis Leuckart, en ont fait les premiers la supposition."

Lühe (1906), in a short note in his account of the blood parasites in Mense's 'Handbuch der Tropenkrankheiten,' has changed the name of this form from *Trypanosoma eberthi* to *Spirochœta eberthi,* owing to the presumed absence of a free flagellate end to the undulating membrane. We think, however, that if Lühe had himself seen this form he would not have placed it among the Spirochætes. It is rather interesting to note that in spite of the numerous references to this common parasite in later literature, and its triple change of name up to the present, Eberth appears to have been the only observer who had actually seen it.

Methods.

Most of the stained work was done on wet films fixed either with Flemming or corrosive-acetic. The films were stained either with iron hæmatoxylin, hæmalaun, Delafield's hæmatoxylen, Giemsa, or Twort. The three first all gave good results.

General Conditions in the Cœca and Rectum.

During the course of this preliminary work we have examined the cæcal and rectal contents of 14 fowls of various ages and at various stages of digestion, and have met with the flagellate parasites of four types, A, B, C, D.

Before, however, proceeding to the description of these forms, it would be well to consider shortly the varying conditions which these parasites have to withstand in the course of digestion. Unfortunately, very little seems to be known as to the part played by the cæca in the metabolism of the bird, and apparently much the same statement may be made as regards the conversion of the fluid intestinal contents into the more solid fæces in any animal. The body temperature of birds is given by Tigerstedt as lying between 39°·4 to 43°·9 C. The temperature of one of our fowls measured in the axilla was 39°·5 C., which is possibly rather low.

The condition of the cæcal content can vary very greatly; in what we have termed the normal cæcal condition the cæca are full of a light brownish fluid containing a large number of small gas bubbles. (In one Irish hen there was an enormous development of gas, and a strong smell of sulphuretted hydrogen; the presence of the sulphuretted hydrogen was possibly correlated with the presence of a rounded plump bacterium found in very large numbers in this hen.)

The most characteristic feature of the bacterial flora in this stage of digestion is a very active vibrio which has been met quite high up the intestine. When the cæca are in this condition, the rectum is frequently empty, and if it is full the contents, though hard, are largely made up of fibres and husks of corn. In what we term the rectal condition of the cæca, the cæca are filled with a dark firm mass; the rectum then may be filled with the same substance, or it may be empty, in which case the animal has probably recently defecated. In the transitional stages between the normal and rectal conditions the bacterial flora undergoes a marked change, the vibrio becomes much rarer, and large numbers of cocci and long slender bacteria are met with. It is evident that in the course of this change of the nature of the cæcal contents, very complicated physical and chemical factors may be at work, not only owing to the action of the walls of the alimentary canal itself but also to that of the bacteria. To single out only one of these factors, the question of the changes in the osmotic pressure of the surrounding medium of the parasite becomes a very important one.

It is a general practice for workers on the intestinal protozoa to carry out extended observations in a solution of sodium chloride, isotonic with the blood, and as regards the fowl, Hamburger, in his recent work on 'Osmotischer Druck und Ionenlehre,' gives two determinations: on p. 176 the figures 0·45 to 0·417 per cent. of sodium chloride are mentioned as the result of plasmolytic methods on blood corpuscles. On p. 458 he gives 0·591 to 0·605 per cent. sodium chloride as a figure arrived at by the freezing-point method. We used a tap water solution of 0·7 per cent. NaCl, but we think that if one regards the events in the course of absorption by which the liquid contents of the cæca and intestine are converted into the practically solid fæces, it becomes difficult to believe that this solution can at best represent more than one out of a series of many stages.

The Flagellate Parasites.

The flagellate parasites found in the cæcum, as has been stated above, can be divided into four groups, A, B, C, D, of which we only need here particularly to consider A, B, and C. D is a sharply marked type, found in small numbers on two occasions, with an anterior and a posterior trailing flagellum, which can be coiled round the body. It is of very small size, roughly half the size of the smallest A form seen, and of approximately torpedo shape.

The chief difficulty in dealing with the remaining three groups, A, B, C, is that although large numbers of individuals can be found in which the characters of each group are sharply marked, yet numerous transitional forms are to be seem. Under these circumstances we do not feel inclined, until we have obtained a great deal more evidence from artificially infected chicks raised in an incubator, to decide definitely between the two alternatives of considering A, B, C as various forms of a mixed infection or as stages of a single life cycle. The small amount of evidence we have at present points clearly to a transition from A to B, and we have observed some cases of which the most natural explanation would be to regard A, B, C as stages in one life cycle.

A. *Trypanosoma eberthi*, Kent. Plate 8, fig. 1.—This is the form which we are inclined to believe Eberth had before him when he wrote his description of *Trypanosoma eberthi*. It is characterised by a rather elongated body, a very well marked undulating membrane, along the edge of which a flagellum runs from the anterior end of the animal to end freely at the posterior end. The living animal, when seen on a cold stage in salt solution, is easily picked up by the rippling movement of the undulating membrane. In the living animal the nucleus can be readily seen as a rather cone-shaped light area containing some small granules lying in the anterior region of the animal. Along the base of the membrane a well marked line can be seen, and in its neighbourhood there is always one and sometimes two rows of blocks. In a suitably placed animal an axostyle, apparently resembling that described in Trichomonas, can be seen. In preparations stained with hæmalaun and eosin, the nucleus is readily recognisable, and a fairly large round body is seen at the anterior end at the origin of the flagellum ; it is probably the kinetonucleus. The line along the base of the membrane stains blue, while the blocks and edge of the membrane take up the eosin very vividly. In hæmalaun preparations which have not been stained with eosin, the blocks and the edge of the membrane may stain blue.

B. *The B Form* (Trichomonas *Condition*). Figs. 2 and 3.—The B form may be described as a typical Trichomonas of variable size, apparently resembling the form described by Wenyon from the mouse. Usually the body is more massive than in the A form, from which it is easily distinguished by the presence of three long conspicuous flagella arising from the anterior end.

In all other points, nucleus, axostyle-line, and blocks, B agrees with A. In living forms, two types of movement have been observed: one closely resembles that of A, the undulating membrane being in active motion, while the anterior flagella trail passively. The second type of movement is characterised by the activity of the anterior flagella, which strike out in front of the animal. In the films stained with hæmalaun and eosin, the anterior flagella, which are longer than the body, are very conspicuous.

C. *C Form* (Monocercomonas *Condition*). Figs. 4 and 5.—This form is roughly egg-shaped. On the blunt extremity there is a well marked cytostome, in the neighbourhood of which four long flagella rise. The body is usually filled with food vacuoles containing bacteria, and the animal is quite active even on the cold stage. The nucleus in the unstained forms can sometimes be seen as a rather highly refractile spherical mass near the anterior end, and there is no trace of an undulating membrane. In stained forms there is no trace of the blocks or line; the nucleus is a spherical object with the chromatin condensed in the membrane, though in some cases there may be a large internal karyosome.

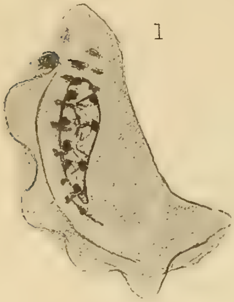
Possible Transitional Stages.

In addition to these forms, small, rounded, generally motionless forms were met with in which the typical nucleus and blocks of the A form were seen. Besides these rounded forms, far more elongated forms were found with a similar nucleus and the blocks; these showed an indication of an undulating membrane in the active motion during life. In these elongate forms a well marked axostyle was seen. If we now return to the forms A and B, the similarity between them in all other points except the absence of the three anterior flagella in A is so great that it almost amounts to identity. On one occasion we saw a living B form with an undulating membrane and posteriorly directed flagella at 12.45 P.M.; at 1.25 P.M. a distinct split was seen along the middle of one of the three flagella. It is tempting to suppose that the change from A to B consists in a splitting off of a free flagellum from the undulating membrane, this flagellum splitting again to form the three anterior flagella of the later stage. This would appear to explain the difference between the two types of B movement mentioned above, the forms with posteriorly directed flagella representing the early stages of this transition from the A to the B form. The differences between B and C are at first sight very marked, but it is interesting to note that C forms, with the rudimentary undulating membrane, a line, and a nucleus approximating to the B type, were met with.

Infection.

Every fowl which we examined was infected by one or other of these flagellate forms, usually in large numbers, as is shown in the following table.

Fowl.	Date.	Locality.	Condition.	Cæcal contents.	Rectal contents.	Flagellates.
I	15.4	Great House, Abergavenny	2 years old; fat	Light brown liquid, with gas bubbles; vibrio abundant	Darker; more solid in small quantity	Spirochæte present, A, B, C.
II	17.4	Holley's Farm, Abergavenny	Old hen	Dark and of an almost earthy consistency; numerous bacteria and cocci	Full of consistent matter	A, B, D.
III	19.4	Limavady, County Derry	Old hen; very fat	Light brown fluid; numerous gas bubbles; H_2S; plump bacteria numerous	Same as cæcum	A, B, C.
IV	20.4	Holley's Farm, Abergavenny	Old hen; fat	Cæcal contents dark and firm	Almost empty.........	B, C.
V	20.4	Llanfoist	Chick; 60 hrs. after hatching	Cæcal contents dark and firm	Ready to defecate ...	C.
VI	20.4	Llanfoist	Old hen	Brown fluid contents	Rectum empty	A, B.
VII	21.4	Great House, Abergavenny	Old hen; very fat	Firm and dark	Recently defecated	B, C. Numerous cysts.
VIII	24.4	Llanfoist	Chick; 10 days	Firm and dark	Just about to defecate	C.
IX	24.4	Limavady, County Derry	Old hen; very fat	Brownish fluid	Dark solid mass, largely composed of husks	A, B, C.
X	24.4	Llanfoist	Chick; 10 days	Brownish fluid	Empty	C.
XI	25.4	Limavady, County Derry	Old hen; very fat	Brownish fluid	Dark solid mass, mostly husks	A, B, C.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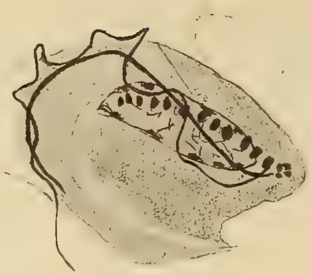
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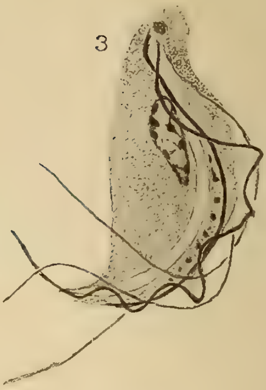
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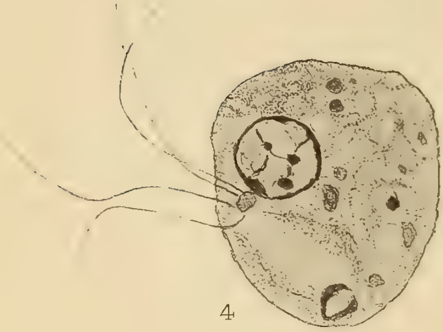
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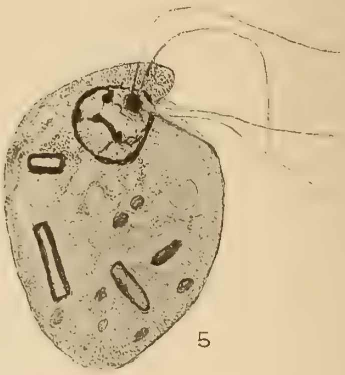
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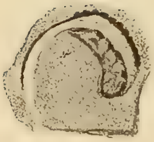
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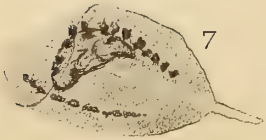
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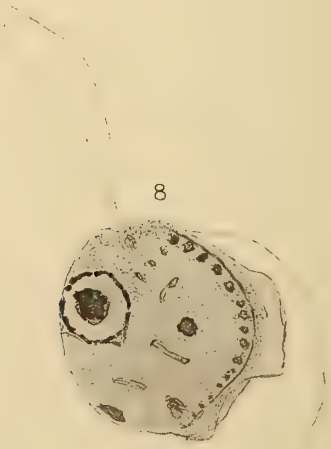
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Infection is probably only effected by food contaminated by fæces containing cysts of the parasites. We propose in the course of the summer further investigation in connection between these interesting forms upon artificially infected chicks hatched out in an incubator.

DESCRIPTION OF FIGURES.

[PLATE 8.]

The figures are drawn at a magnification of about 4500 diameters. Zeiss 2 mm. 250 mm. tube, and 12 oc.

FIG. 1.—A form (*T. eberthi*), showing nucleus, undulating membrane, blocks, and axostyle. In typical A forms the free flagellum is longer.

FIGS. 2 and 3.—B forms (Trichomonas), showing three flagella, undulating membrane, staining line under membrane, axostyle and nucleus.

FIGS. 4 and 5.—C form (Monocercomonas), showing four flagella, cytostome, nucleus, and food vacuoles.

FIG. 6.—Small rounded non-motile A form.

FIG. 7.—A form, small, showing blocks and axostyle and nucleus, also indication of undulating membrane.

FIG. 8.—Form possibly intermediate between B and C.

The Possible Ancestors of the Horses living under Domestication.

By J. C. Ewart, M.D., F.R.S., University of Edinburgh.

(Received May 15,—Read June 24, 1909.)

(Abstract.)

During the later part of the Nineteenth Century it was generally taken for granted—(1) that " the seven or eight species of Equidæ now existing are all descended from an ancestor of a dun colour more or less striped ";* (2) that the common ancestor of the living horses, asses, and zebras was connected by a single line of descent with the four-toed " fossil " horses of the Eocene period ; (3) that the domestic horses are descended from a Pleistocene species characterised by large molars with a long anterior internal pillar, a large, heavy head, and coarse limbs ; (4) that in various parts of Europe and Asia domestic races increased in size and were improved in make, speed, and disposition as a result of artificial selection and favourable surroundings.

On the Continent it seems to be still generally assumed that the domestic breeds have descended from a single species,† but in England and America many naturalists now believe—(1) that domestic horses have sprung from several wild species, connected by several lines of descent with three-hoofed species of the Miocene period ; and (2) that while some of the wild ancestors were adapted for living in the vicinity of forests and upland valleys, others were adapted for a steppe, plateau, or desert life.

Of possible ancestors of the domestic breeds, the following may be mentioned :—*Equus sivalensis, E. stenonis, E. gracilis* (Owen's *Asinus fossilis*), *E. namadicus, E. fossilis,* and *E. robustus.* These species mainly differ in the teeth, size and deflection of the face, and in the bones of the limbs. In the first three species, the grinding surface of the anterior internal pillar (a fold of enamel on the inner surface of the cheek teeth) of the premolars and first molar is short, in the last premolar, pm. 4, it may only be one-third the length of the crown; in the second three species, the anterior internal pillar is long—at least half the antero-posterior length of the crown.

One of the ancestral types (*E. robustus*) was broad browed and had a short face, almost in a line with the cranium ; another (*E. sivalensis*), also broad

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* Darwin, 'Animals and Plants,' vol. 2, p. 17.

† The latest suggestion is that domestic horses are the descendants of *Equus fossilis*, Rütimeyer, a Pleistocene species closely allied to the wild horse of Mongolia—*E. przewalskii.*

browed, had a long, tapering, strongly deflected face; a third (*E. fossilis*) had a long, narrow face, not so strongly bent downwards as in *E. sivalensis*; and a fourth (*E. gracilis*) had a fine, narrow, but only slightly deflected, face.

In *E. gracilis* the middle metacarpal (cannon bone) was so slender that the length was seven and a-half times the width, while in *E. robustus* the length of the metacarpal was sometimes only five and a-half times the width.

Of these possible ancestors, the first three occur in Pliocene deposits, the second three have only hitherto been found in Pleistocene deposits.

Equus sivalensis, of the Siwalik deposits of Northern India, is the oldest true horse known to science (*i.e.* the oldest one-hoofed horse with long (hypsodont) molars), and, as it measured about 15 hands, it is the largest of the Old World "fossil" horses. This ancient Siwalik horse was characterised by long, fairly slender limbs, and a long, tapering face, deflected to form an angle of nearly 20° with the base of the cranium. In addition to having a large head, a convex profile, and long limbs, *E. sivalensis* seems to have been characterised by a long neck, high withers, and a tail set on so high that the root was well in front of the point of the buttock. Nothing is known of the ancestors of the horse which suddenly made its appearance in Pliocene times amongst the foot hills of the Himalayas, but it may be safely assumed that *E. sivalensis* very decidedly differed from *Pliohippus*, the small "fossil" horse of the late Miocene and the early Pliocene deposits of America, from which some believe all the recent Equidæ are descended.

It used to be said that *E. sivalensis* could not be regarded as an ancestor of domestic horses because of the shortness of the anterior pillar of the cheek teeth. I find, however, that in some modern horses the anterior pillars are decidedly shorter than in *E. sivalensis*, and that in some of the short-pillared domestic horses the face is nearly as strongly deflected on the cranium as in *E. sivalensis*. There is hence no longer any reason for assuming that this ancient Indian species had no share in the making of domestic breeds. But in the absence of a large and representative collection of skulls of domestic horses it is impossible to say which modern breeds are indebted to the large-headed, long-limbed race which in Pliocene times frequented the area to the east of the Jhelum River, now occupied by the Siwalik Hills.

Mr. Lydekker thinks *E. sivalensis*, or some closely allied race, "may have been the ancestral stock from which Barbs, Arabs, and Thoroughbreds are derived." When more skulls are available for study and when the phases through which equine skulls pass during development and growth have been worked out, it will probably be ascertained that broad-browed horses with a prominent interorbital region—a forehead convex from side to side as well as from above downwards—and a long, tapering, strongly deflected face have in great part descended from a species closely allied to *E. sivalensis*, but that horses with a broad flat forehead, and the face short and nearly in a line with the cranium, are at the most only remotely related to *E. sivalensis*.

Further inquiries will probably also show that some Indian breeds as well as some of the unimproved races of Central Asia (*e.g.*, certain long-faced Kirghiz horses with a sloping forehead and long ears) in many of their points agree with *E. sivalensis* of the Pliocene deposits of Northern India.

The second possible ancestor mentioned is *Equus stenonis* of the Pliocene deposits of Europe and North Africa. In a typical specimen of this species, with the teeth in an intermediate state of wear, all the anterior pillars of the premolars and molars are shorter than in *E. sivalensis*, while in a specimen with the teeth well worn the longest pillar may be only one-third the length of the grinding surface of the crown. At no age are the pillars of the molars more than half the length of the crown. Whether the face was long and tapering and strongly deflected in *E. stenonis* has not yet been determined, but from the limb bones collected it is evident that the horse with short-pillared molars, which in Pliocene times frequented the valley of the Arno, sometimes reached a height of nearly 15 hands.

It is generally supposed *E. stenonis* either became extinct towards the close of the Pliocene age or was modified to form varieties with long-pillared molars. It is conceivable that some of the descendants of *E. stenonis* acquired long-pillared molars, but it by no means follows that all the Pleistocene horses of Europe with the anterior pillars more than half the length of the crown are related to or derived from *E. stenonis*—some of them may have been the descendants of *E. namadicus*. Be this as it may, horses with teeth of the *E. stenonis* type existed in the south of Scotland during the first and second centuries, and horses with short-pillared cheek teeth are still in existence. In some of the skulls from the Roman fort at Newstead the anterior pillar of the third and fourth premolars only measures 9 mm., which is only about half the length of the pillar in *E. namadicus* and other "fossil" Pleiostocene species. Further, in one of the first century Newstead skulls the first premolar is as large as in *E. stenonis*, and the face (as broad and long as in *E. sivalensis*) forms an angle of 18° 6′ with the cranium.

In all probability further inquiries will show that the short-pillared species (with metacarpals as long but somewhat thicker than in *E. sivalensis*) widely distributed over Europe and North Africa in Pliocene times played an important part in the making of Shires and other heavy modern breeds.

The only other possible ancestor dealt with in this contribution is the one to which I have given the name *Equus gracilis*. Owen arrived at the conclusion that Pleistocene horses "had a larger head than the domesticated races," and that even in small varieties the teeth were nearly as large as in a modern cart horse.

Having come to these conclusions, it is not surprising that when it fell to his lot to describe small equine molars from the drift overlying the London clay and from a cavernous fissure at Oreston, near Plymouth, he decided that they could not belong to a true horse and (on the assumption that they belonged to an extinct ass or zebra) formed for them the species *Asinus fossilis.*

In addition to the small second and third molars described and figured by Owen, there is in the British Museum a small first molar from Oreston. The anterior pillars of the second and third Oreston molars are more than half the length of the crown, as in horses of the "forest" type, but the pillar of the first molar, m. 1, from Oreston is only about one-third the length of the crown as in *Pliohippus* and *E. stenonis.*

Except in size, the small teeth from Oreston and other Pleistocene deposits bear little resemblance to the molars of asses or zebras, but they are practically identical in enamel foldings as well as in size with the molars of a small (12·2 hands) slender-limbed horse in the possession of the Auxiliaries who garrisoned the Roman fort at Newstead in the south of Scotland about the end of the first century.

In addition to small equine teeth, the Devonshire Pleistocene deposits have yielded a small slender metacarpal. This metacarpal (from Kent's Cave, near Torquay), is 220 mm. long and 30·25 mm. wide—the length is hence 7·27 times the width, as in fine-boned Arabs.

As might have been anticipated from a study of the teeth, the Kent's Cave metacarpal belongs to a very much finer-limbed race than the small horse of the "elephant" bed at Brighton. On the other hand, the Kent's Cave metacarpal very closely agrees with the metacarpals of the small Newstead horse.

This small first-century horse in teeth and limbs agrees with Exmoor, Hebridean and other ponies of the Celtic type, *i.e.* with ponies characterised by a small fine head, large eyes, slender limbs, five lumbar vertebræ, and by the absence of the hind chestnuts and all four ergots.

It hence follows that the small equine of the English Pleistocene (Owen's *Asinus fossilis*), instead of being an ass or a zebra, is a true horse, which in the metacarpals, as in the "pillars" of the premolars and first molar, differs but little from Pliohippus of the late Miocene and early Pliocene American deposits.

Remains of a small horse with teeth and limbs like *Equus gracilis* (*Asinus fossilis,* Owen) have been found in the Pliocene deposits of Italy and France and in the Pleistocene deposits of France and North Africa. The Italian and Auvergne slender-limbed horse has generally been regarded as a small variety of *E. stenonis.* By Pomel and other palæontologists the French variety was known as *E. ligeris,* while the North African variety, named *Equus asinus atlanticus* by Thomas, was regarded by M. Boule as closely allied to, if not the ancestor of, zebras of the Burchell type.

The slender metacarpals from the valley of the Arno and Auvergne so closely resemble the Kent's Cave metacarpal, and the teeth from Perrier and Puy de Dome in France and Lake Karar in Algiers so closely resemble the small teeth from Oreston, that *E. ligeris* and *E. asinus atlanticus* may be regarded as varieties or races of *E. gracilis.*

There are good reasons for believing that *E. gracilis* varied to form a northern and a southern variety. Remains of a slender-limbed northern race have been found in deposits belonging to the Neolithic, Bronze, and still later ages in Britain and on the Continent. At the present day the purest representative of this northern variety is the Celtic pony. Hence this northern variety may be known as *Equus gracilis celticus.*

Remains of a slender-limbed southern variety have not yet been found in recent deposits in North Africa, but fine-limbed ponies without ergots and hind chestnuts are sometimes met with in the south of France, and slender-limbed horses without hind chestnuts—horses almost certainly of North African descent—are occasionally met with in the West Indies and Mexico. In the French, and still more in the wartless ponies of Mexico, the limbs are longer than in the Celtic ponies, the coat is finer, the mane less full, and the "taillock," so well developed in the northern variety, is very small. As the southern variety in all essential points agrees with Prof. Ridgeway's fine bay horse of North Africa (*E. caballus libycus*), it may be known as *E. gracilis libycus.*

Slender limbs and the absence of ergots and hind chestnuts are apparently as distinctive of members of *E. gracilis* as an upright mane and the absence of hind chestnuts are distinctive of asses and zebras. Hence, when, as a result of crossing varieties possessing four ergots and four chestnuts, slender-limbed individuals without ergots and hind chestnuts appear in any area, it may be assumed that the horses of that area include *E. gracilis* amongst their ancestors.

From inquiries made and from crossing experiments it has been ascertained that ponies of the Celtic type occur in the Faroe Islands and Iceland, in the Western Islands and Highlands of Scotland, in the west of Ireland, in Wales, Exmoor, and the New Forest, and in Norway and Finland.

Further crossing experiments have made it evident that the yellow-dun fjord horses of Norway are mainly a blend of the Celtic and "forest" types, that the Shetland ponies, though usually having the conformation of the "forest" or *E. robustus* type, are in part of Celtic origin, and that some of the mouse-dun Tarpans of the Russian steppes are a nearly equal blend of the Celtic and *E. przewalskii* types.

Prof. Ridgeway arrived at the conclusion that in the fine bay horse of North Africa there is a frequent tendency to stripes on the back, legs, shoulders, and face, to a blaze on the forehead and to white "bracelets." Experiments made with four types of Arabs and with Russian, Mongolian, Indian, and Borneo ponies, English, Irish, Iceland, and Norse ponies support the view that the Pleistocene ancestor of the modern slender-limbed ponies with short-pillared molars was of a yellow or bay-dun colour with a narrow dorsal band and bars on the legs, but had neither "bracelets" nor a blaze. As stripes are most numerous on broad-browed horses, they have probably in most cases been inherited from ancestors of the *E. robustus* or *E. sivalensis* types.

As to the part played by *E. gracilis libycus* in forming domestic breeds, nothing very definite has been made out: Prof. Ridgeway says all the improved breeds of the world are a blend in varying degrees of the bay horse of North Africa with thick-set, slow, dun and white horses of Europe and Asia allied to *E. przewalskii.* A number of hybrids bred at Woburn by the Duke of Bedford afford little, if any, evidence in support of the view that Barbs, Arabs, or Thoroughbreds include amongst their ancestors horses of the Prejvalsky or "steppe" type.

Slender-limbed horses with a wide flat forehead and a nearly straight profile appear to be a blend of *E. gracilis libycus* (Ridgeway's *E. caballus libycus*) and horses of the *E. robustus* ("forest") type, while slender-limbed strains with a fine narrow face, a well set-on tail, and a mane that clings to the neck, probably most accurately reproduce the variety of *E. gracilis* which in prehistoric times inhabited North Africa.

Hillhousia mirabilis, *a Giant Sulphur Bacterium.*

By G. S. West, M.A., D.Sc., F.L.S., and B. M. Griffiths, B.Sc.

(Communicated by J. Bretland Farmer, F.R.S. Received July 7, 1909.)

[Plate 9.]

The organism which forms the subject of this paper has been under the observation of one of us for a number of years, but its true nature was not immediately recognised. It has been found in various parts of England and Ireland since 1892, always occurring in very stagnant pools and marshy bogs. Until quite recently it was only found very sparingly amongst Algæ, Infusoria, etc., generally in situations where there was much decomposing organic matter. During the past winter, however, the organism has been found in abundance in the mud of the shallower part of an old pool in Worcestershire. This has enabled us to make cultures of it, and to make a study of its general biology and structure. We have named it *Hillhousia mirabilis.**

The cells are solitary but gregarious, occurring freely among other organisms and numerous small particles of decaying organic matter. They secrete very little or no mucus, even in a culture, so that colonies are not built up.

They are for the most part shortly cylindrical, with hemispherical ends, and are usually straight. Some individuals exhibit a very slight curvature, and others may be observed which are slightly attenuated towards each extremity. The cells are from one and three-quarters to three and a-quarter times as long as broad.

For a unicellular bacterium the dimensions of this organism are phenomenally large. *The diameter of the cell* varies from 20 to 33 μ, and *averages about* 26 μ. *The length* varies from 42 to 86 μ, and *averages about* 60 μ.

In its normal healthy condition the cell is packed with globules of oily amorphous sulphur of various sizes, the largest of which have a diameter of about 10 μ (Plate 9, figs. 1 to 3). These globules are very crowded, and, when isolated, are seen to be somewhat irregular, although rounded (fig. 17). Their refractive nature gives the organism almost a black appearance under the microscope, but a pale yellow or yellow-green colour is quite obvious on careful inspection by transmitted daylight.

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* The generic name "*Hillhousia*" has been given in honour of Prof. W. Hillhouse, the retiring Professor of Botany in the University of Birmingham, to whom both authors owe much in the way of kindly advice and criticism.

Owing to the large quantity of sulphur they contain, the cells are very heavy, sinking in the water almost like sand-grains.

The cells are motile, exhibiting slow oscillatory and rolling movements. *The organism is a peritrichous bacterium with several hundred short cilia* disposed all over the exterior of the cell-wall. The cilia can be seen immediately on fixation either with a 5-per-cent. carbolic acid solution or with a 40-per-cent. formalin solution. The action of these reagents results in a cessation of the movements of the cilia in from 10 to 20 seconds, during which period many of them are thrown off and become disintegrated. Some of these cilia exhibit a contractile or wriggling movement after having been thrown off, indicating that the contractility of the cilium is not dependent upon attachment at the base. The cilia are plainly observed by the use of the above reagents, but only within a few seconds of the moment of fixation (figs. 15 and 16).

The cilia can also be clearly seen in active movement by examining the living organism by means of the Zeiss or Leitz dark-ground illuminator. It is likewise possible to demonstrate the presence of cilia by immersing the living organisms in a drop of indian ink. The cilia appear as a halo around the cell, and the minute particles of ink can be seen in rapid motion on the edge of the halo. This movement is of a different character from the Brownian movement of the minute particles in the water. The tiny particles of ink can be seen to be lashed about and driven into small eddies.

Cell-division is relatively slow, and in no observed instance was a single division completed in less than 24 hours, the time occupied in many cases being as much as 48 hours.

The Sulphur Globules.—The globules of sulphur are enormously larger and much more densely packed than in any of the other known sulphur bacteria. They are dissolved by glacial acetic acid,* by boiling in a solution of magnesium sulphate; and also by prolonged boiling in potassium chlorate. They turn almost black on boiling in ferrous sulphate; and a brownish-black precipitate is found in the cells after boiling in lead acetate. A 2½-per-cent. solution of commercial formalin entirely removes the globules. Carbon bisulphide penetrates the cell-wall only with difficulty, but after penetration quickly dissolves the sulphur.

When the living organisms in a pure condition are placed in distilled water the sulphur globules disappear in about 48 hours, the sulphur being oxidised in the process of respiration. The oxidation of the sulphur can best be demonstrated by allowing a culture to remain undisturbed for several days in a small quantity of water containing minute traces of lime. The organisms gradually lose all their sulphur, and a considerable deposit of granular crystals of calcium sulphate is formed.

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* This was pointed out by Corsini in the case of *Beggiatoa*; *cf.* A. Corsini, "Ueber die sogenannter Schwefelkörnchen die man bei des Familie der 'Beggiatoacæ' antrifft," 'Centralbl. für Bakteriol.,' II, 1905.

By allowing the bacteria to dry on a slide, and then irrigating with water, the sulphur is obtained in small rhombic crystals (fig. 18). Crystals can also be obtained by allowing the formalin solution in which the bacteria have been fixed to slowly evaporate. These do not appear, however, to be crystals of pure sulphur.*

From the general behaviour of the sulphur globules, there are many reasons for supposing that the sulphur is not pure, but exists in some kind of loose combination, possibly with proteid material. The crystals obtained by drying the organisms and then irrigating with water are only formed *outside* the cells. After irrigation the colloidal sulphur, or sulphur-compound, passes through the wall of the cell, without causing the latter any injury, and crystallisation of the sulphur takes place in the surrounding medium.

Cultures.—Like other sulphur bacteria, *Hillhousia* will not grow on gelatine or agar.† We have obtained the best cultures in tap water containing minute traces of sulphuretted hydrogen.

Straining the mud through coarse muslin enables one to obtain almost all the bacteria, mixed only with various small living organisms, finely divided flocculent organic matter, and small sand-grains. The great weight of the bacteria can now be utilised to obtain a pure collection, as it is possible to remove the other organisms by means of a fine pipette, while the large sulphur bacteria and the small sand-grains remain as a sediment. If this sediment be now transferred to a watch-glass it is possible to separate the major portion of the sand-grains by a judicious tapping of the glass while held in a slightly inclined position. In this way the bacterium can be obtained mixed only with very minute sand-grains. We have not yet succeeded in obtaining a culture of the bacterium entirely free from these tiny fragments of silica, although some method of chemotaxis might possibly solve the difficulty. The culture should now be shaken up at intervals, and very small quantities of sulphuretted hydrogen water added every few days.

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* After several hours' treatment with 40-per-cent. formalin, the individual bacteria are found to be encrusted with a thick deposit of radiating crystals. On the addition of more water to the solution the crystals are dissolved. If weaker formalin is used, the crystals are never formed, as the substance (some compound of sulphur) is dissolved as fast as it is produced.

† Winogradsky, in 'Ann. de l'Institut Pasteur,' 1889, pp. 49 and 50, has stated his inability to obtain cultures of *Beggiatoa*, *Thiothrix*, *Chromatium*, etc., on solid culture media.

Such a culture will thrive for a time, although the multiplication of the organism is relatively very slow.

The organism thrives best when the flocculent organic matter, after straining, is allowed to remain in the water. Under these circumstances the bacterium remains healthy, and can be kept for a very long time without any addition of sulphuretted hydrogen water.

Experiments in obtaining pure cultures are still proceeding, and discussion of this part of the investigation is for the present deferred.

Keeping the organism in the original mud in which it is collected, without constant change of water, proves unhealthy and ultimately fatal. This is due to the accumulated excess of sulphuretted hydrogen in the water, which causes the bacteria to lose their sulphur. The addition of a strong solution of sulphuretted hydrogen to a culture also causes a solution of the sulphur and the death of the organisms.

Light is unnecessary for the perfect growth of this bacterium, cultures thriving as well in complete darkness as in diffuse light.

In mass and by reflected light a culture presents a greyish-white appearance.

Cytological Structure.—Formalin has been found the most useful fixing reagent on account of the fact that the sulphur globules are removed at the same time. A $2\frac{1}{2}$-per-cent. solution of commercial formalin will completely remove the sulphur in the course of a few hours.*

Individuals fixed in this way show *a network of protoplasm* which occupies the interstices between the sulphur globules, the position of the latter being indicated by the large clear spaces (figs. 5 and 19). *Embedded in the protoplasmic network are numerous minute granules* of very variable size. (These are well shown in fig. 19.)

The *cell-wall* is highly resistant to reagents, but becomes much more permeable after the organism has been dried. It contains no cellulose, and stains yellow on the addition of iodine. It dissolves only with difficulty in sulphuric acid, does not dissolve in an ammoniacal solution of cupric hydrate, and in many ways it is suggestive of fungus-cellulose. Its great resistance to reagents is probably due to the presence of a considerable proportion of chitin.

On the addition of 5-per-cent. carbolic acid to the living organisms, the cell-wall swells up and becomes lamellose, indicating that it is not of homogeneous structure (figs. 13 and 14). There appear to be several firm layers, with intervening layers which become somewhat gelatinous on the addition of carbolic acid. The innermost layer is a firm one, but the outermost layer, which can only be demonstrated by special methods, is apparently gelatinous.*

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* The removal of the sulphur is probably brought about by the small quantity of free formic acid present in commercial formalin.

Nothing of the nature of a definite nucleus is present in the cell, but as the protoplasmic network includes many conspicuous granules, very careful tests for nucleins have been made.

Staining has given very indefinite results. Erythrosin and methylene blue were of little use. Safranin and carbol-fuchsin were found the most useful stains, and double staining with safranin and gentian-violet gave good results. Carbol-fuchsin stains the protoplasmic network very well, but in no instances were the included granules distinctly brought out. Cover-glass preparations gave much better results than any other method. The granules of the network do not appear to have an affinity for any of the stains used, and they cannot be regarded as chromatin granules.

Microchemical tests for nucleo-proteids have been carefully repeated many times, using cultures of the organism fixed in 2½-per-cent. commercial formalin. As stated before, by this means the sulphur globules are removed and the fixed protoplasmic network can be experimented upon. Owing to the impermeability of the cell-wall it was found necessary to allow the operations to extend over a considerable period.

Treatment with concentrated sodium carbonate removed fully nine-tenths of the granules, while the network remained clear and refractive (fig. 21).

A 10-per-cent. salt solution removed a large proportion of the granules, probably about five-sixths of them, and the network was again left clear except in the central part of the cell, where there appeared to be a concentration or shrinking together of the protoplasmic strands (figs. 10 and 11). For this reaction, and also the previous one, the cultures were exposed to the reagent for rather more than 14 days.

Treatment with dilute potash (5 per cent.) gave a variety of results due probably to the degree of penetration of the potash in different individuals. In most cases, after about 10 days, the granules were for the most part dissolved, and the network to a great extent disorganised (fig. 12).

A number of cultures were treated with acidulated pepsin-glycerin, and in these cases the network was for the most part digested, whereas the majority of the granules remained unchanged. Where the network had only partly disappeared, the granules had the appearance of highly refractive beads strung on fine threads (fig. 20).

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* There is evidence of this even in the active state of the organism, as the cells have a tendency to stick to the bottom of the vessel in which the culture is growing.

The above tests, taken collectively, furnish evidence which goes far to prove that a considerable proportion of the granules present in the general protoplasmic network consist of nucleo-proteids.

Lastly, the bacterium was tested for phosphorus. A considerable quantity of a culture (pure except for very minute grains of silica) was incinerated on platinum foil, and kept at a red heat for several minutes. The ash was then treated in small tubes with a reagent consisting of 10 c.c. of nitric acid to 1 gramme of ammonium molybdate, and kept at a temperature of 50° C. for a week, after which period numerous minute crystals of an intense yellow colour were present in all the slides prepared. These crystals belong to the cubic system, and there is every reason to regard them as crystals of ammonium phospho-molybdate. Slides of the reagent only, kept for the same period at 50° C., showed no trace of such yellow crystals.

From this test we conclude that phosphorus is present in the bacterium, and therefore that some nucleo-proteid is present.* The previous tests indicate that this nucleo-proteid is in the form of small granules in the protoplasmic network, and the staining proves the granules are not particles of chromatin.

Thus, Hillhousia *is a very primitive unicell in which chromatin has not been developed, and the particles of nucleo-proteid (possibly of the nature of linin) are scattered evenly through the whole protoplasmic network of the cell.*

Although the cytological structure of *Hillhousia* can be studied with comparative ease, it must not be assumed that other and less easily investigated bacterial cells have a similar structure. The sulphur bacteria may be of a low type, and it is quite probable that among the various known groups of the Schizomycetes there are bacteria in which the cytological structure is of a somewhat higher order.

The present investigation is only of a preliminary character, as much work yet remains to be done in obtaining pure cultures, and in further working out the cytology of the organism.

Summary.

Hillhousia mirabilis is a sulphur bacterium of giant proportions, and is much the largest solitary bacterium which has so far been discovered. Its average length is about 60 μ and breadth about 26 μ.

The organism is a peritrichous bacterium with a large number of short

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* Galeotti (in 'Zeitschr. für physiol. Chemie,' vol. 25, 1898, p. 48) has definitely demonstrated the occurrence of a nucleo-proteid in certain bacteria.

cilia. It occurs among decaying organic matter in the mud of shallow freshwater pools.

Each individual contains a protoplasmic network in the wide meshes of which large globules of sulphur (probably not pure, but in loose combination with proteid material), are located. The network includes numerous small granules, a considerable proportion of which consist of some nucleo-proteid. None of them are chromatin granules.

The cell-wall is firm and has great powers of resistance to reagents. It is not homogeneous, and 5-per-cent. carbolic acid demonstrates its lamellose character.

The multiplication of the organism is relatively slow, one division occupying upwards of 24 hours.

EXPLANATION OF PLATE.

FIGS. 1—16, each × 500; FIGS. 17—21, each × 1000.

FIGS. 1—3.—Drawings of living specimens of *Hillhousia mirabilis* to show the dark refractive sulphur globules practically filling the whole cell.

FIG. 4.—Specimen kept in tap water for one week. The sulphur globules have been almost entirely used up in respiration.

FIG. 5.—Individual after being in a 2½-per-cent. solution of commercial formalin for several days. The sulphur is completely removed and the protoplasmic network becomes very obvious. Note the numerous granules in the network.

FIG. 6.—Outline of a curved cell. These are rarely observed, the great majority of the cells being straight.

FIGS. 7—9.—Outline of three distinct specimens showing three of the principal stages in simple cell-fission. In fig. 9 the constriction is almost complete.

FIGS. 10, 11.—Two individuals after treatment for 14 days with 10-per-cent. NaCl. Many of the granules in the network have been removed, and there is a decided contraction or shrinking of the central parts of the network.

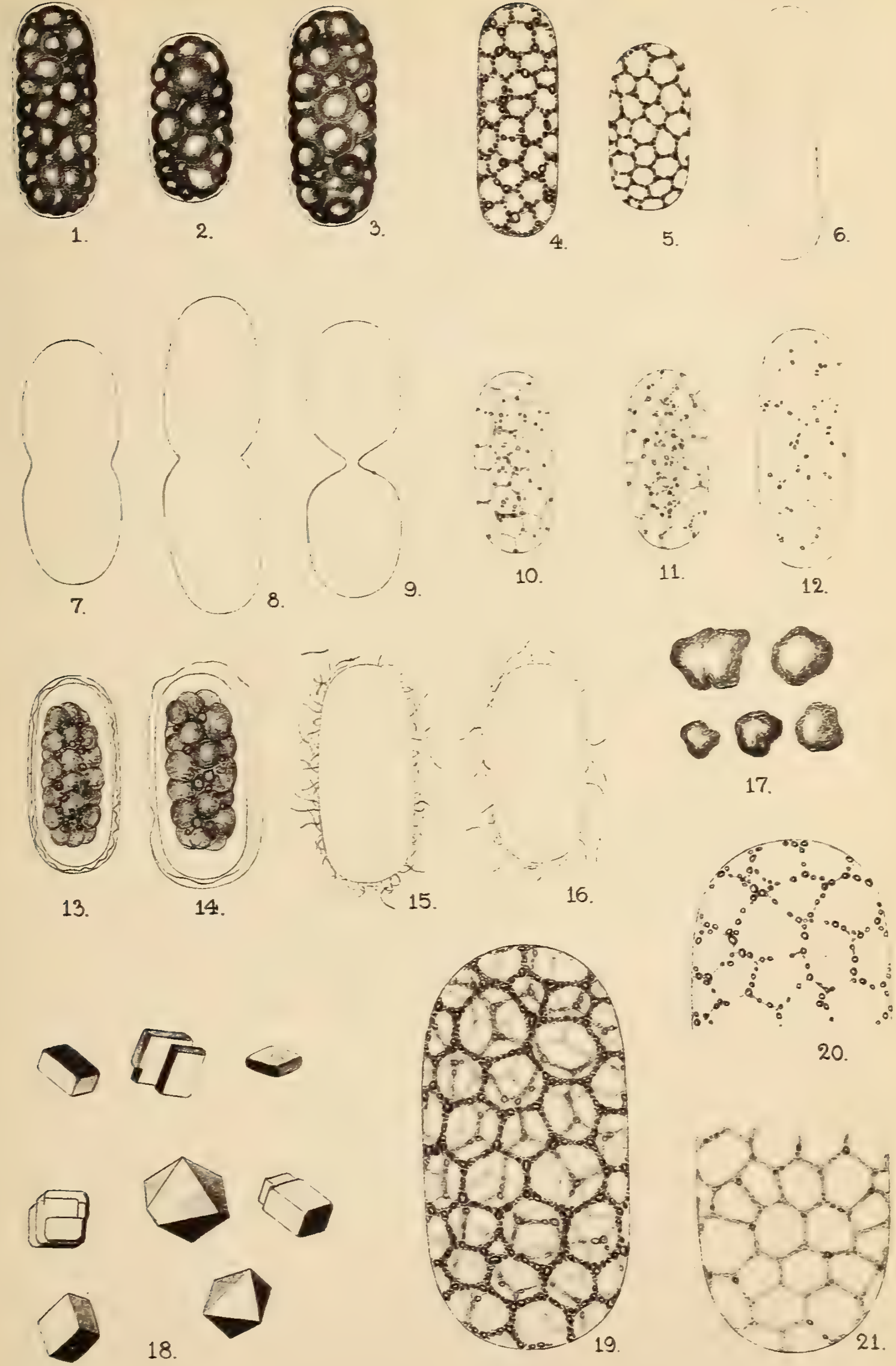
FIG. 12.—Individual after prolonged treatment with 2-per-cent. KOH. The protoplasmic network is largely disorganised, and a large number of the granules have been dissolved.

FIGS. 13, 14.—Two cells after treatment for about 15 minutes with 5-per-cent. carbolic acid. The cell-wall exhibits a lamellation, and the sulphur globules have coalesced into a central irregular mass.

FIGS. 15, 16.—Two cells immediately on treatment with 40-per-cent. commercial formalin. The numerous short cilia are very readily observed for a brief period, and many of them can be seen to be thrown off into the surrounding liquid.

FIG. 17.—Isolated sulphur globules, showing their irregular form, obtained by crushing the living cell.

FIG. 18.—Small crystals (rhombic prisms and rhombohedra) of sulphur obtained by allowing the living organisms to completely dry up. These crystals are formed *outside* the organisms, the colloidal sulphur passing through the cell-wall to the outside after irrigating with water.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HILLHOUSIA MIRABILIS, WEST & GRIFFITHS.

FIG. 19.—Single specimen after treatment with $2\frac{1}{2}$-per-cent. commercial formalin, showing both the parietal and the more internal portions of the protoplasmic network. The granules are shown only in the upper parietal portion of the network.

FIG. 20.—One extremity of an individual after treatment for 14 days with acidulated pepsin-glycerin. Only the granules of the surface network are represented; these stand out very clearly, but the protoplasmic network itself has been for the most part digested.

FIG. 21.—One extremity of an individual after treatment for 14 days with a concentrated solution of Na_2CO_3. Only the surface network is represented. The protoplasmic network remains clear and distinct, but most of the granules have been dissolved.

The Development of Trypanosoma gambiense *in* Glossina palpalis.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captain A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908.)

(Received July 5, 1909.)

[PLATES 10 AND 11.]

The following experiment is so complete in itself that no apology is offered for publishing it by itself. In 1903 the Sleeping Sickness Commission of the Royal Society came to the conclusion that the carrying of infection from a sleeping sickness patient to a healthy person by the *Glossina palpalis* was a mechanical act, and required no previous development of the parasite within the fly. The Commission also held that the power of transferring the disease was lost to the fly 48 hours after it had fed on an infected person.

Koch and Stuhlmann, in German East Africa, described developing forms in *Glossina*, but did not succeed in infecting healthy animals by the injection of these forms.

Kleine, in German East Africa, at the end of 1908, succeeded first in showing that *Glossina palpalis* could convey *Trypanosoma brucei* some 50 days after the fly had fed on an infected animal.

It seems, at first, strange that this fact should have escaped notice for 15 years, and can only be accounted for by assuming that it is an event of the rarest for a fly to be found which fulfils the unknown conditions necessary for the development of the trypanosomes in its interior. If we assume that it is only one fly in a hundred or in a thousand in which this development takes place, then the difficulty of observing the phenomenon can be understood.

Take the following experiments, for example:—

Table I.—Flies caught in an Infected Area, kept for some days, and then fed on Healthy Animals.

Trypanosoma brucei—Glossina morsitans.

Expt.	Place.	Observer.	No. of flies fed.	No. of times flies fed.	No. of days before infection or under observation.	Result.
210	Zululand	Bruce	5	32	64	Negative.
242	„	„	30	11	56	„
232A	„	„	50	15	34	„

These experiments seemed to show that if flies caught in a highly infected district, into which a horse could not be taken even for a few hours without contracting nagana, are kept without food for a few days—say three to five—they are then incapable of conveying infection. This appeared to be a strong proof that the duration of infectivity in the fly was a short one, since, if this were not the case, 1 of the 85 flies ought to have been in a condition capable of infecting, having, of course, been infected at some previous date in the "fly country." It may be repeated, that these flies were caught in a most highly infected district, so that if *Glossina morsitans* can remain infective for 50 or 60 days, 1 at least of the 85 ought to have been in the condition which made it capable of conveying the disease.

This development of the trypanosomes in the fly is strikingly like what occurs in the test-tube with Novy's medium. A thousand tubes are inoculated with *Trypanosoma brucei*: the trypanosomes all appear to die off, but 20 days afterwards a peculiarly resistant individual is found in one tube of the thousand, who has adapted himself to the new enviroment, and soon multiplies into myriads. What it is which enables this particular individual to adapt itself to such altered conditions is unknown. It is the merest speculation to call it a sexual act and pick stout forms as females and slender forms as males.

Again, because this late development of the trypanosomes enables a particular fly to remain infective for 100 days, or even possibly for the remainder of its life, it by no means follows that this is the usual method of infection. The mechanical transference of the disease is proved up to the hilt, and for every case which falls a victim to the rare late-infected fly, a thousand must be infected by direct mechanical transference.

SUMMARY OF THE EXPERIMENT WHICH FORMS THE SUBJECT OF THIS PAPER.

Before describing at length the experiment which forms the subject of this paper, we may summarise it as follows:—

1. On March 5, 1909, 60 *Glossinæ palpalis* caught on the lake shore were placed in two cages, 30 in each. The flies were fed on two infected monkeys for 2 days. They were then starved for 72 hours to get rid of mechanical transference. The following 5 days they were placed on a healthy monkey, and every successive period of 5 days, or thereabouts, on a fresh monkey, up to 86 days, when the experiment came to an end. The result was, that the first two monkeys remained healthy, but that all the following monkeys, up to 75 days, became infected with *Trypanosoma gambiense.*

2. If 7 days be deducted for the incubation period, then the flies first became infected 18 days after their first feed on an infected animal.

3. There is some evidence that among the 60 flies only 1 was infective. Fifty-four days after the beginning of the experiment each cage was placed on a separate monkey. Up to that time both the cages of flies had been fed on the same animal. Cage A contained, after 54 days, 11 flies. Cage B, 4 flies. Cage A continued to infect monkeys for 21 days more, making a total of 75 days. Cage B did not infect. Again, as was natural, the flies gradually died off during the experiment, and as each fly died it was carefully dissected and examined for trypanosomes. Not a single trypanosome of any kind whatever was seen in any dissected fly up to 75 days, when a fly died in Cage A which was found to be swarming with trypanosomes similar to *Trypanosoma gambiense.* After the death of this fly, Cage A ceased to be infective, and when the experiment was stopped the remaining flies were killed off and dissected, but among them not a sign of a trypanosome could be seen. In the same way the flies remaining in the non-infective Cage B were examined, with a similar negative result.

4. Here follows an interesting and unique observation. A tiny drop of fluid taken from the gut of the 75-day fly injected under the skin of a monkey gave rise to Sleeping Sickness after an incubation period of eight days. This, so far as we are aware, is the first time this has been recorded.

5. It will be seen from the detailed experiment that the flies were starved for three days between several of the experiments. This, of course, was to get rid of the fallacy of mechanical transference.

6. It may be said that perhaps these monkeys became infected by some other means than the flies in the cage—for example, by other biting flies, or by contact. To this it may be answered that there are more than 200

monkeys under observation here, sick and healthy. They are all examined twice a week, but during the last eight months not a single case of accidental infection has taken place.

DETAILS OF THE EXPERIMENT.

Experiment 663.

To ascertain if development of *Trypanosoma gambiense* takes place in the interior of *Glossina palpalis,* and if so, how long does the fly remain infective.

March 5, 1909.—Two batches of *Glossina palpalis* caught on the Lake shore, consisting of 30 flies in each batch, were fed on monkeys, Experiments 568 and 214, whose blood contained numbers of *Trypanosoma gambiense.*

March 6.—The flies again fed as on the 5th, to ensure that as many as possible should get a feed of the infected blood. Nearly all the flies fed on one or other occasion. The flies are kept in a moist atmosphere at 22° C.

The following table gives the principal details of the experiment:—

Table II.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Mar. 5	—	Flies fed on infected monkey			
6	1	„ „ „			
7	2	Flies starved 72 hours			
8	3	„ „			
9	4	Fed on Monkey 579		–	
10	5				
11	6				
12	7				
13	8				
14	9	„ „ 651		–	
15	10				
16	11				
17	12				
18	13				
19	14	„ „ 652	+		
20	15				
21	16				
22	17				
23	18				
24	19	„ „ 653	+		
25	20				
26	21				
27	22				
28	23				
29	24	„ „ 654	+		
30	25				
31	26				
Apr. 1	27				
2	28				

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Apr. 3	29	Fed on Monkey 655	+		
4	30				
5	31				
6	32				
7	33				
8	34	" " 672	+		
9	35				
10	36				
11	37				
12	38				
13	39	" " 722	+		
14	40				
15	41				
16	42	Starved for 72 hours			
17	43				
18	44	Fed on Monkey 727	+		
19	45				
20	46				
21	47				
22	48				
23	49				
24	50				
25	51				
26	52	Starved for 76 hours			
27	53				
28	54	Cage A fed on Monkey 735 ...	+		
29	55				
30	56				
May 1	57				
2	58	" B " " 736 ...		–	
3	59	Starved for 74 hours			
4	60				
5	61	Cage A fed on Monkey 749 ...	+		
6	62				
7	63				
8	64				
9	65	" B " " 748 ...		–	
10	66	Starved for 72 hours			
11	67				
12	68	Cage A fed on Monkey 765 ...	+		
13	69				May 13.—Flies remaining in Cage B killed and dissected.
14	70				
15	71				
16	72	" B " " 764 ...		–	
17	73	Starved for 72 hours			
18	74				
19	75	Cage A fed on Monkey 848 ...		–	May 19.—Fly 866 found dead in Cage A and dissected. Did not feed on Monkey 848.
20	76				
21	77				
22	78				
23	79				
24	80	Starved for 72 hours			
25	81				
26	82	Cage A fed on Monkey 911 ...		–	
27	83				
28	84				Expts. 848 and 911 healthy on 7th June, 1909. Remaining flies killed and dissected.
29	85				
30	86				
31	87	Experiment stopped.			

Remarks on the Experiment.

Everyone will agree that this is a most interesting experiment. It is evident that a single infected fly did all the mischief, and by good luck this fly was detected. Captain A. E. Hamerton, D.S.O., had charge of the experiment at first, and on his leaving Mpumu about the beginning of May, it fell to Sergeant A. Gibbons, Royal Army Medical Corps. Both are to be congratulated on the results, which are the outcome of care and thoroughness. Captain F. P. Mackie had the good fortune to dissect the fly which did the injury, and which will be fully described later.

INCUBATION PERIOD.

From the experiment may be drawn the incubation period in monkeys bitten by a late-infected fly.

It is remarkable how regular this is in those monkeys which gave a positive result. This shows how very infective Fly 866 was. Apparently each time it bit it infected.

The following table gives the period of incubation in each case:—

Table III.

Date.	Experiment.	Flies first fed.	Trypanosomes appeared in blood.	Number of days before trypanosomes appeared in blood.
1909.		1909.	1909.	
March 19	652	March 19	March 30	11
„ 24	653	„ 24	April 2	9
„ 29	654	„ 29	„ 6	8
April 3	655	April 3	„ 13	10
„ 8	672	„ 8	„ 15	7
„ 13	722	„ 13	„ 20	7
„ 18	727	„ 18	„ 24	6
„ 28	735	„ 28	May 5	7
May 5	749	May 5	„ 11	6
„ 12	765	„ 12	„ 17	5

Leaving out the first experiment, 652, as it is doubtful as to the exact day Fly 866 became infective, this gives an average incubation period of seven days. It would therefore appear that Fly 866 probably infected each animal on the first day it bit it, showing how dangerous such an infected fly is.

DESCRIPTION OF THE *Glossina palpalis*, FLY 866, WHICH WAS DISSECTED 75 DAYS AFTER HAVING FED ON A MONKEY WHOSE BLOOD CONTAINED *Trypanosoma gambiense.*

Experiment 866.

May 19, 1909.—Dissected a *Glossina palpalis*, which was found dead to-day in Cage A of Experiment 663. On removing the viscera by the usual method, the mid-gut was seen to be of a pale salmon-pink. A small quantity of its contents, examined in the fresh condition, was found to contain enormous numbers of trypanosomes. The tube of this part of the intestine was absolutely crammed with active, seething masses of these flagellates. In regard to the other parts of the fly, nothing was seen in the proboscis. In the proventriculus one trypanosome only was found. The salivary glands contained large numbers of altered-looking trypanosomes, the fore-gut many large stout forms, with bright granules. The crop was empty and showed nothing. The Malpighian tubules, hind-gut, and proctodæum also were drawn blank.

In addition to examining these organs in the fresh condition, smears were made and stained. The examination of these stained specimens gave the following results:—

The salivary glands.—These had been carefully removed before the intestine was opened, and therefore had no chance of being fouled. As will be seen from the coloured drawing (Plate 10, fig. 1), the trypanosomes found in these glands differed from those seen in the intestine. The bodies are very irregular in shape, and contain, besides a reddish-stained nucleus, dark deeply-stained coarse chromatin granules. The other cell contents remain unstained. Free chromatin granules and flagella are to be seen scattered over the field. Sometimes the bodies are definitely pear-shaped, with a flagellum coming from the narrow end, and rarely a more definite trypanosome shape can b seen; but never a true trypanosome.

[It is a matter of deep regret that an inoculation experiment was not made with an emulsion of part of the salivary glands.]

The fore-gut.—The fore-gut contained many trypanosomes. The cytoplasm stains a pale blue, and the nucleus a reddish-purple. The micronucleus is not distinctly seen in some of the trypanosomes, but when it is, it is always distinctly posterior to the nucleus. The protoplasm contains many coarse darkly-stained chromatin granules. The undulating membrane is less marked than in the normal blood trypanosome, and the flagellum, which usually springs from a micronucleus-like body, is less deeply stained (Plate 11, figs. 6—13).

The mid-gut.—The mid-gut contained innumerable trypanosomes of the *gambiense* type. Some are dividing, and all have a well-marked nucleus and micronucleus, the latter at or near the posterior extremity. The protoplasm contains many chromatin granules, and an undulating membrane and flagellum are present (Plate 10, figs. 6—16). Many groups, or rosettes, composed of 15 to 20 individuals, occur, the flagella pointing outwards (Plate 11, fig. 1).

The *proboscis, proventriculus, thoracic gut, crop, hind-gut,* and *Malpighian tubes* contained no trypanosomes.

The most interesting thing in this description of the examination of Fly 866 is the condition of the salivary glands. How these trypanosome-like bodies, or derivatives of trypanosomes, got into them is a mystery, and we will content ourselves at present with merely placing the bare fact on record until the salivary glands of similarly infected flies are examined.

There is one fallacy which might be pointed out. It is assumed that Fly 866 became infected on the first or second day of the experiment. It is possible that it became infected when feeding on the fifth day on an animal which showed trypanosomes in its blood a day or two later. This, however, is unlikely, as no other fly showed trypanosomes on dissection.

In order to make the story more complete, on Plate 10, figs. 1—5, is represented the *Trypanosoma gambiense* from the blood of one of the monkeys on which the flies were fed at the beginning of the experiment, and on Plate 11, figs. 2—5, are shown *Trypanosoma gambiense* from the monkey which became infected from the contents of the mid-gut of Fly 866.

PROPORTION OF INFECTED FLIES TO NON-INFECTED IN NATURE.

In the experiment under consideration it is seen that, in artificially-infected flies, only 1 in 60 showed the phenomenon of late infectivity. In nature the proportion must be less, as many of the flies, in many places at least, can never have fed on an animal whose blood contained *Trypanosoma gambiense.*

That there can be but few under natural conditions Table IV shows. The table is made by subtracting the flies fed on the animal during the last seven days, before trypanosomes were found in the blood, this being the incubation period, from the total number. The experiments consist in catching tsetse flies in the infected area, bringing them to the laboratory and placing them straightway on healthy animals.

The first two experiments were made with *Trypanosoma brucei* and *Glossina morsitans,* and it would appear from them that 104 and 108 flies

Table IV.—Table to show Probable Number of Naturally infected Flies per thousand.

Expt.	Place.	Observer.	No. of flies fed before infection took place.	Result.		Probable No. of naturally infected flies per thousand.
				Positive.	Negative.	
Trypanosoma brucei—Glossina morsitans.						
225	Zululand	Bruce	104	+		9·6
236	„	„	108	+		9·2
Trypanosoma gambiense—Glossina palpalis.						
94	Uganda	Bruce and Nabarro	89	+		11·2
130	„	Bruce, Nabarro, and Greig	850	+		1·2
131	„	„ „	506	+		1·9
136	„	Nabarro and Greig	723		–	
228	„	Greig and Gray	866	+		1·2
301	„	„ „	2299		–	
45	Leopoldville	Dutton, Todd, and Hannington	457		–	
46	„	„ „	552		–	
128A	River	„ „	25		–	
139	„	„ „	262		–	
141	„	„ „	52		–	
182	Kasongo	„ „	211		–	
198	„	„ „	2659	+		0·4
203	„	„ „	1789		–	
213	„	„ „	717		–	
52	Uganda	Bruce, Hamerton, Bateman, and Mackie	41		–	
214	„	„ „	3284	+		0·3
568	„	„ „	178	+		5·6
571	„	„ „	850	+		1·2
53*	„	„ „	21		–	
612	„	„ „	615	+		1·6
674	„	„ „	2315	+		0·4

* Animal died.

were used respectively before an infective one was found. This perhaps explains why Bruce's 85 flies failed to infect.

In the experiments with *Trypanosoma gambiense* and *Glossina palpalis* the average is 2·5 per thousand. It is, of course, impossible to tell how many of these positive experiments were infected by mechanical transference or by a late-infective fly; but, in any case, the proportion is small. If this were not so, all the native population of the Lake shore, and most of the Europeans in Uganda, would long ago have been blotted out.

DESCRIPTION OF PLATES.

Plate 10.

Smear preparation of salivary glands of *Glossina palpalis*, Experiment 866, stained Giemsa, showing irregularly shaped trypanosomes, with unstained protoplasm, reddish-coloured nuclei, and deeply stained chromatin granules. Note the chromatin granules scattered singly about the field, each surrounded by a pale area, fig. 1. × 2000.

Normal *Trypanosoma gambiense* from monkey, Experiment 568, on which the flies were fed at the beginning of the experiment, figs. 2, 3, 4, and 5. × 2000.

Trypanosomes from the mid-gut of infected fly, Experiment 866, figs. 6—16. × 2000.

Plate 11.

Rosette form from the mid-gut, fig. 1. × 2000.

Trypanosoma gambiense from the blood of monkey, Experiment 868, into which a tiny drop of the contents of the mid-gut of Fly 866 had been injected, figs. 2—5. × 2000.

Trypanosomes from the fore-gut of Fly 866, stained Giemsa, figs. 6—13. × 2000.

A Note on the Occurrence of a Trypanosome in the African Elephant.

By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908.)

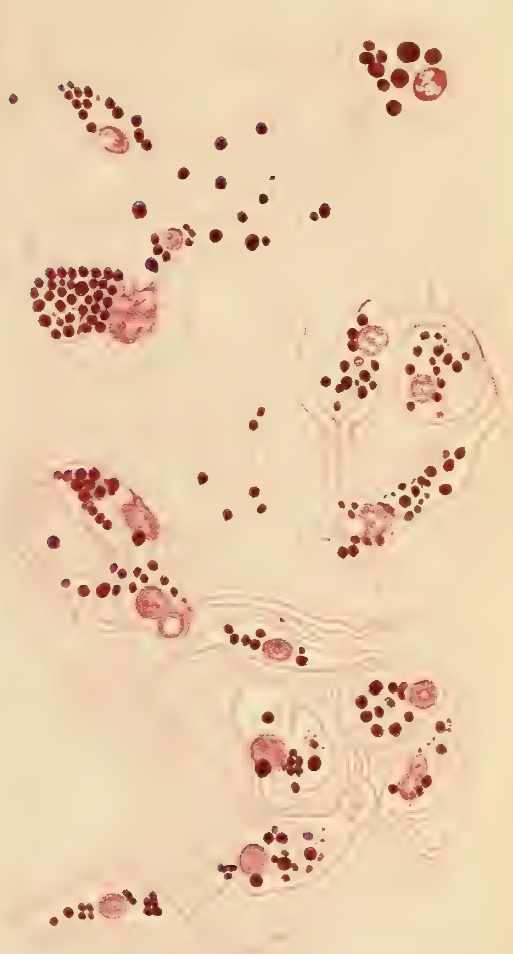
(Received July 5, 1909.)

[Plate 12.]

As trypanosomes have never been reported as having been observed in the blood of the African Elephant, the Commission thought it would be of interest to note this observation.

In Laveran and Mesnil's book on trypanosomes, translated by Nabarro, on p. 261 it is stated that "the occurrence of Surra (*Trypanosoma evansi*) in elephants in India and Burmah is practically proved. In this connection we have only the statement of G. H. Evans that, in 1893, 14 out of 32 elephants died of the disease in Burmah." The year 1893 is almost prehistoric for trypanosomes. At that time observers had even failed to distinguish between the common rat trypanosome—*Trypanosoma lewisi*—and that of Surra. It may well be, then, that Evans was mistaken in his diagnosis of the species causing this large mortality in elephants.

The African elephant, in whose blood this trypanosome was found, was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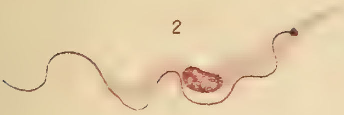
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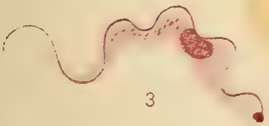
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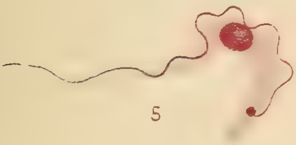
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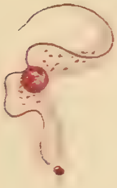
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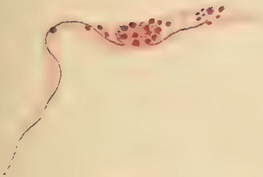
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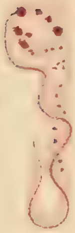
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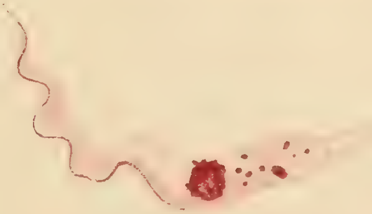
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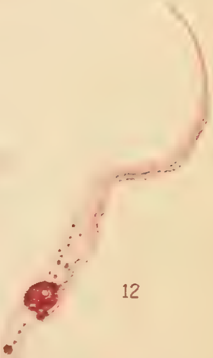
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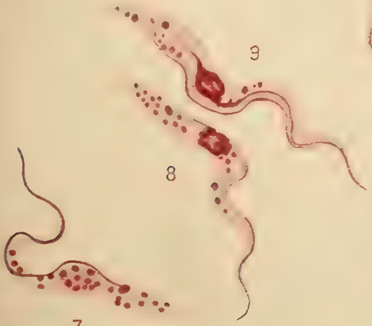

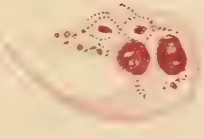
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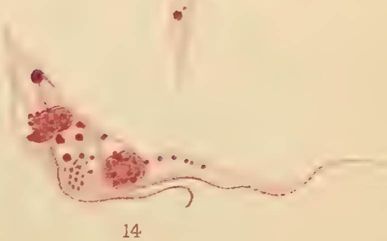
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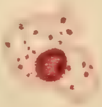
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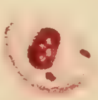
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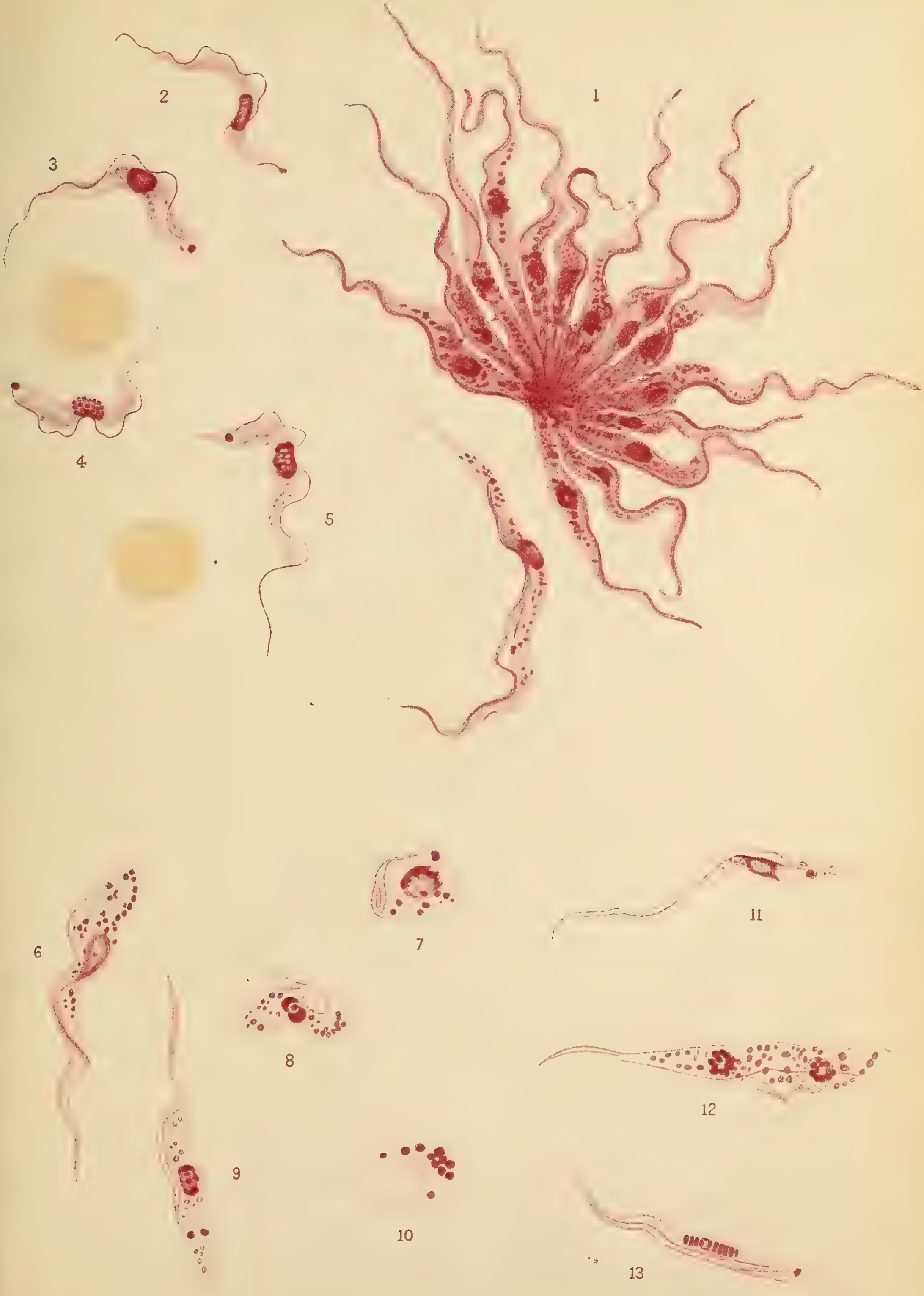
M. E. Bruce del.
West, Newman chr.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M. E. Bruce del. West, Newman chr.

shot by Mr. L. C. Lea-Wilson, of the Uganda Company, Limited, at a spot two miles from the eastern shore of Lake Albert, near Ngogole, about 31° 10′ E. lat. and 1° 30′ N. long. It is to be regretted that none of the blood was injected into a dog, donkey, or ox, in order that a fuller study of this trypanosome might have been made. As it is, all the material available are a couple of smears made by Mr. Lea-Wilson and sent to the Commission.

Morphology of the Trypanosome of the Elephant.

Method of Fixing and Staining.—The two slides received from Mr. Lea-Wilson were fixed in osmic acid vapour and alcohol, stained in Giemsa, and decolorised in orange tannin.*

Length.—For method of measurement see the same paper, p. 16. As will be seen from the coloured plate, which was drawn by Sergeant Gibbons, R.A.M.C., this trypanosome is of medium size. The average length of 18 individuals is 18·5 microns: maximum 21, minimum 15.

Breadth.—On an average the breadth at the thickest part is 3 microns.

Shape.—This trypanosome is of the *Trypanosoma brucei* type, inasmuch as it has a well-developed undulatory membrane and free flagellum. As will be seen from the drawing (Plate 12), one noteworthy feature it has is the uniformity in size and shape of the different individuals. The posterior end is blunt, or conical, reminding one somewhat of the head of a seal, with the bulging micronucleus for an eye. The body thickens as far as the middle, when it gradually tapers away to the anterior end.

Contents of Cell.—The protoplasm is clear and particularly free from granules.

Nucleus.—The nucleus is compact and sharply defined from the neighbouring protoplasm. In shape it is round, or oval, and often lies nearer the anterior extremity than the posterior. Its length averages 2 microns.

Micronucleus.—The micronucleus is small, round and distinct. It is situated close to the posterior extremity, and often appears to bulge above the surface.

Undulating Membrane.—The undulating membrane is well developed and thrown into well-marked folds.

Flagellum.—The flagellum stains deeply. It runs from the micronucleus along the edge of the undulating membrane, beyond which it projects as a free flagellum for some 5 or 6 microns.

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* *Vide* 'Roy. Soc. Proc.,' Series B, vol. 81, p. 16.

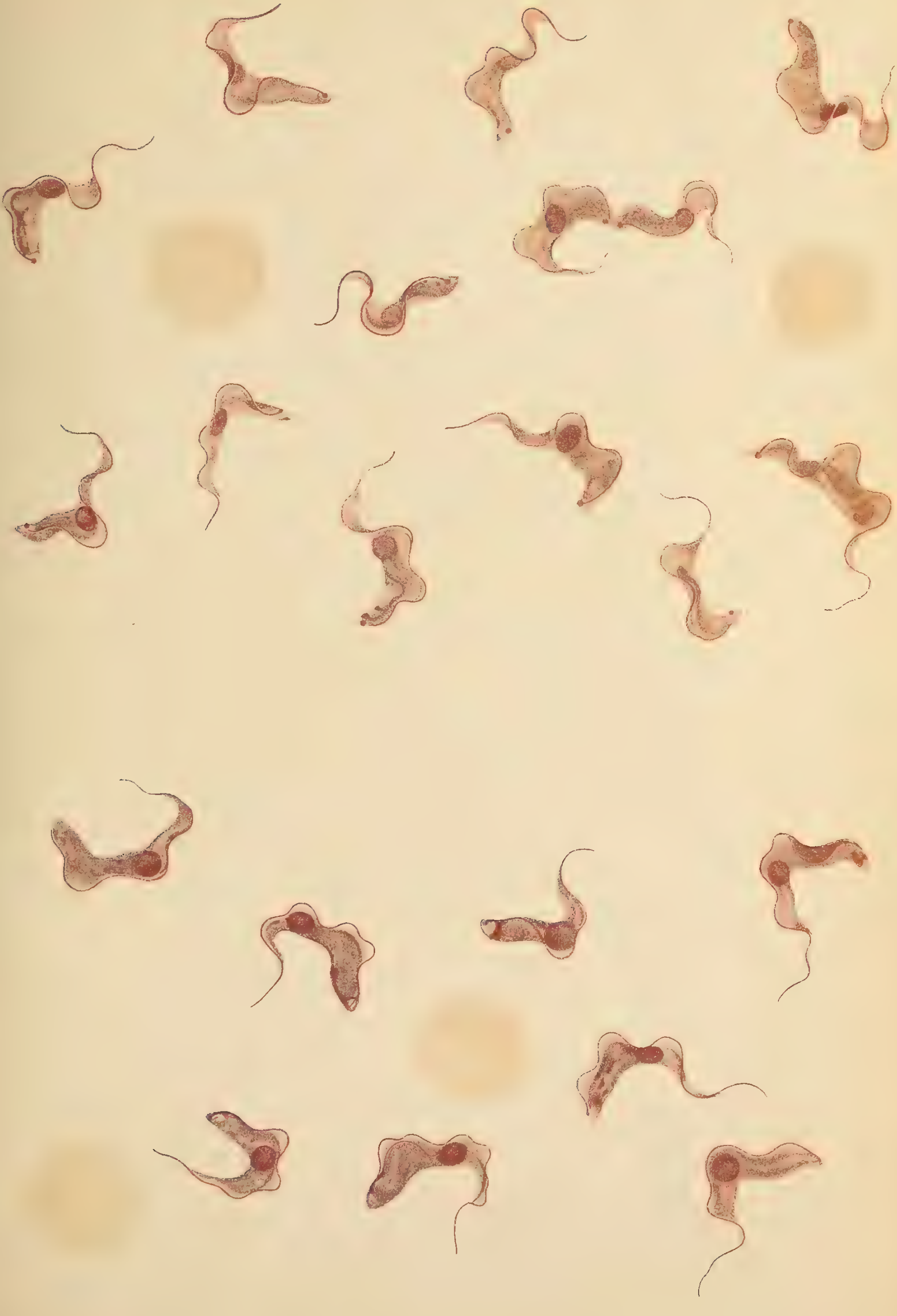
Conclusions.

In our present state of knowledge it seems impossible to name trypanosomes from their form alone. We were, however, much surprised, a short time ago, by Sir John McFadyean separating with ease *Trypanosoma brucei* from *Trypanosoma evansi*. If this can be done for such closely related species, surely it should be possible to do it for all. To assist to this end it would be well if observers would adopt one method of fixing, staining, and measuring. In the 'Third Report of the Wellcome Research Laboratories,' Khartoum, facing p. 30, there is a coloured plate of trypanosomes, stated to have a magnification of 1000. On measuring one of them it is found to have a magnification of between 2000 and 3000. Then, again, many of the trypanosomes depicted are dividing forms, which is misleading.

The method of measuring must also make a difference. For example, in Laveran and Mesnil's book the length of *Trypanosoma brucei* in the rat is given as 26 to 27 microns, whereas by our method of measuring the average length of 20 individuals is 22·8 microns: maximum 25, minimum 20.

The trypanosome of the elephant has an average length of 18·5 microns: maximum 21, minimum 15, a well-developed undulatory membrane and free flagellum. The trypanosomes with free flagella are *Trypanosoma brucei, cazalboui, evansi, gambiense, pecaudi,* and *soudanense.* It probably is neither *Trypanosoma cazalboui* nor *pecaudi,* on account of its well-developed undulating membrane and uniform size. Under the circumstances it is impossible to decide as to its identity with *Trypanosoma brucei, gambiense,* or *soudanense,* but if a guess were hazarded then it would be *Trypanosoma soudanense.*

Until the nature of this species is better known we propose to name it *Trypanosoma elephantis.*



Sergt Gibbons, R A M.C., del.

West, Newman chr.

The Ferments and Latent Life of Resting Seeds.

By JEAN WHITE, M.Sc., Victorian Government Research Scholar.

(Communicated by D. H. Scott, F.R.S. Received March 3,—Read March 18, 1909.)

This subject was suggested to me by Prof. Ewart as an outcome of his long series of experiments on the longevity of seeds.* For the most part, I have confined my attention solely to the seeds of cereals as being of the greatest importance in agriculture.†

The scheme proposed for the carrying out of these investigations necessitated the procuring of old grains; this was not an easy matter, but after several months I was very fortunate in having seeds forwarded from the Agricultural Department, Victoria; Dookie Agricultural College, Victoria; the Chamber of Commerce, South Australia; and the Hawkesbury Agricultural College, New South Wales. For these I am much indebted to Messrs. J. Knight, H. Pye, and W. Potts, whom I now take the opportunity of thanking for the trouble they have taken.

Many of the seeds obtained from the Agricultural Department of Victoria were travelling samples, and had been in the possession of the department for from 8 to 10 years, but whether they had been harvested the same season or the previous one to that in which they were put up is not exactly known. The greater number of the specimens sent from South Australia and New South Wales were accompanied by information as to the exact date of their harvesting.

The oldest grains available were samples of wheat received from South Australia which had been stored for 21 years.

The old specimens of barley, oats, and rye were amongst those previously referred to which were obtained from the Agricultural Department, Victoria, and whose minimum age must be at least 8 to 10 years. In all the above cases, certain of the different samples of grains had completely lost all their power of germination, so that they were exactly what I required in order to be enabled to carry out one section of the work. I was not quite so fortunate as regards the maize, for the oldest seeds in my possession were grown only 4½ years ago, and had only partially lost their germinating power.

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* 'Proceedings of the Royal Society of Victoria,' vol. 21 (New Series), August, 1908.

† The whole of the work in the following paper has been carried out in the Botanical Laboratory of Melbourne University under Prof. Ewart's supervision, who has also critically tested and verified certain experiments and written the summary at the end of the paper. The expenses of the work were defrayed from the Research Scholarship and Apparatus Fund of the Victorian Government.

The paper is divided into sections, the first of which relates to the relative germinating powers of grains of various ages, and as far as possible obtained from different sources.

The second section deals with the connection (if any exists) between the age of the seeds and the persistence of their enzymes, with special reference to any possible co-relation between the germinating power retained by the stored grains and their enzymes.

The third section is a detailed account of experiments performed on the seeds at temperature extremes, more especially concerning their germinative capability and their enzyme reactions, as in the previous section.

The fourth section is a brief account of experiments concerning the respiratory activity of certain seeds in a more or less dried condition and the results obtained therefrom.

1. *Germinating Power of Seeds.*

The germination capacity of all the specimens received was tested, and the data so obtained are shown in the columns below. From 50 to 100 seeds from each packet (the number depending on the quantity of material at my disposal) were sown on damp blotting paper placed in glass basins, which were put under a glass frame in the conservatory to which air had free access. The temperature of the conservatory was kept fairly constant at about 23° C.

The comparative germinating capabilities are given in the following table (A):—

Germination Table A. (Wheat.)

Age.	Percentage germinated.	Place of origin.	Age.	Percentage germinated.	Place of origin.
6 months	100	S. Australia.	8½ years	32	S. Australia.
6 ,,	100	Victoria.	8½ ,,	3	Victoria.
1½ years	100	S. Australia.	9½ ,,	32	S. Australia.
1½ ,,	100	Victoria.	9½ ,,	0	Victoria.
2½ ,,	100	S. Australia.	10½ ,,	28	S. Australia.
2½ ,,	100	Victoria.	10½ ,,	0	Victoria.
3½ ,,	100	S. Australia.	11½ ,,	12	S. Australia.
4½ ,,	100	,,	11½ ,,	0	Victoria.
4½ ,,	92	Victoria.	12½ ,,	4	S. Australia.
4½ ,,	90	New South Wales.	13½ ,,	0	,,
6½ ,,	77	S. Australia.	15½ ,,	0	,,
6½ ,,	42	Victoria.	16½ ,,	2	,,
6½ ,,	39	New South Wales.	17, 18, 19, 20, and 21 years	0	,,
7½ ,,	68	S. Australia.			
7½ ,,	16	Victoria.			

Germination Table B. (Barley, Oats, Maize, and Rye.)

Kind of seed.	Age.	Percentage germinated.	Place of origin.
Barley	1½ years	100	Victoria.
"	2½ "	100	"
"	4½ "	72	"
"	4½ "	54	New South Wales.
"	8½ "	18	Victoria.
"	10½ "	0	"
Oats	1½ "	96	"
"	2½ "	80	"
"	4½ "	68	"
"	5½ "	56	"
"	9½ "	0	"
Maize	6 months	100	"
"	4½ years	60	New South Wales.
Rye	6 months	100	Victoria.
"	4½ years	32	"
"	9½ "	0	"

In those cases in which different samples of Victorian seeds of the same age were experimented with, the average number which germinated is given in the tables. The majority of the seeds were sent in cotton bags, and the method in which they had been stored was not stated. In the case of wheat, however, taking for granted similar conditions of storage, the fact that the South Australian specimens retained their germination capacity for a longer period than the Victorian specimens, and the Victorian specimens retained it longer than those from New South Wales, shows that the drier the climate the longer is the life of the seed. On further reference to the wheat table, it appears that there is a well-marked drop in the germinating power of the grains after about the fourth year, and from thence it descends more or less irregularly, reaching zero in 11 to 17 years, according to the character of the sample and the conditions of storage.

2. *The Relation between the Longevity of Seeds and their Contained Ferments.*

The investigations were carried out with seeds freshly harvested and with stored seeds which had lost the faculty of germination.

The object of these investigations was to determine, as has been previously stated from time to time by different authorities, whether the loss of the germinating power was concomitant with, and caused by, or in any way related to, the disappearance of the capability of enzyme action in the seed.

The seeds used were wheat (*Triticum vulgare*), barley (*Hordeum sativum*), oats (*Avena sativa*), maize (*Zea mais*), and rye (*Secale cereale*).

In every instance, except in the maize, seeds were employed in which the germination capacity was entirely lost, but of the oldest specimens of maize obtainable 60 per cent. of the seeds sowed germinated.

Samples of the same seeds which had been tested for loss of germinating power as described in the preceding section were first tested for the presence of *diastase ferments.*

1. *Diastase.*—The method adopted for the precipitation of the diastase was the same as that described by Darwin and Acton in the 'Physiology of Plants,' p. 305. Ten grammes of the seed were ground to a fine powder in a hand coffee mill, and placed in a bottle containing 100 c.c. of slightly warm distilled water, which was shaken for two hours with the aid of a water-motor rocker.

The mixture was then filtered and the filtrate concentrated at 50° C. under decreased pressure to about half its original volume. To the filtrate was added enough 90-per-cent. alcohol to produce a white flocculent precipitate. This precipitate was separated by filtration, and the filter-paper containing the precipitate was placed in an exhausted desiccator over sulphuric acid during the night. When dry, the precipitate was scraped off the filter-paper by means of a sterilised knife, and dissolved in a small volume of cold, boiled water.

Equal quantities of this aqueous solution were put into each of three test-tubes which had been previously sterilised, and the contents of one tube C were thoroughly boiled. A very thin starch solution was prepared, and, when cool, equal quantities of the starch solution were added to each of the test-tubes B and C and also to a fourth test-tube D. Of the four test-tubes A, B, C, D, A contained only the original aqueous solution of the precipitate. B contained about equal quantities of the aqueous solution of the precipitate and starch solution. C contained the same as B, with the exception that the aqueous solution of the precipitate had been boiled and allowed to cool before the addition of the starch solution. D contained only starch solution. The four test-tubes were placed in a bath at a constant temperature of 50° C. After about one or one and a-half hours the test-tubes were removed from the bath, and the contents of each were tested for the presence of reducing sugar by means of Fehling's test. All through the experiments only 1 drop of No. I Fehling was added to each test-tube and 2 drops of No. II. In every instance, as may be observed from the detailed lists of experiments given below, the presence of reducing sugar was detected in the contents of the test-tube B, while no sign of reduction was noted in the contents of any of the other test-tubes.

The results of these experiments, which were performed with many varied specimens obtained from widely different sources, denote conclusively the presence of a more or less considerable quantity of a *diastase ferment* in the precipitate obtained from the extract of the crushed resting seeds. This ferment was present as such, and not as a zymogen in the resting seed, and was destroyed by boiling, as shown by the experiments performed with the test-tubes C.

The experiments were performed as far as possible in pairs, in one of which freshly harvested grain, and in the other old grain of the same kind, whose germinating power was lost, were tested.

It was impossible to carry out any additional control experiments with the aid of commercial diastase, for this was found in all cases to contain reducing sugar, and it was not found possible with the means at hand to prepare sufficient quantities of pure diastase from the material available.

It has been stated that the artificial addition of commercial diastase to ungerminable or feebly germinable seeds may bring about or increase their germination,* and the idea that ferments are connected with the vitality of seeds is a fairly prevalent one.†

In order to test this statement, samples of such seeds were sown on damp blotting paper, and a little dissolved commercial diastase was added to them. Similar numbers from the same packets of seeds were sown on damp blotting paper and, instead of dissolved diastase, a little plain water was poured over them. In addition, two similar sets of seeds were sown as above, but the seed coat of each seed, both in those treated with dissolved diastase and with water alone, was pierced once with a needle. In this way the entry of the ferment was assured even in the presence of a more or less impermeable integument.

Not all the seeds which were tested for the presence of diastase were thus treated, chiefly owing to the limited quantity of the available material, but sufficient experiments were performed with each kind of seed to ensure accuracy of results.

Effect of Addition of Diastase.—The addition of the dissolved diastase to intact seeds does not materially affect their germinating power, and in no case does it bring about germination in otherwise non-germinable seeds. The presence of external diastase aids the development of bacteria and interferes with the aeration of the seeds, which may be sufficient in some cases to produce a slight lowering of the percentage germination by preventing the germination of feebly germinable seeds.

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* Thompson, 'Garten-Flora,' vol. 45, p. 344, 1896; Waugh, 'Ann. Rept. Vermont Agric. Exp. St.,' 1896—7; 'Science,' N.S., vol. 6, p. 950, 1897; Sharpe, 'Mass. Hatch Exp. St.,' 1901, p. 74.

† Brnyning, Jr., F. F., "Relation entre le pouvoir germinative et l'activité diastatique de graines non-germées"; Albo, G., 'Bull. della Soc. Bot. Ital.,' 1908; 'Archiv des Sci. phys. et nat.,' vol. 25, p. 45.

The effect of pricking the seeds is apparently injurious, for, as a general rule, less of the pricked seeds developed than of the unpricked ones. In addition, samples of all these seeds were tested for the presence of diastase while in the resting condition, 5 to 20 grammes being used for extraction according to Darwin and Acton's method. The precipitate was allowed to act on starch solution for one hour, and then tested for reducing sugar.

Considering the case of the wheat first, as set out in the following tables, it will be observed that while age materially affects the germinating power of the seeds, it does not apparently materially influence the quantity of diastase enzyme present in the seed, nor its activity, at any rate up to an age of 20 years.*

In every instance, strong reduction was produced by Fehling's test, and the variations in the degree of reduction noted in the tables were extremely slight, and might be due to differences between the samples when originally harvested.

The different samples of barley gave closely similar results, and the same applies to the oats, rye, and maize.

The oats exhibited the greatest amount of variation, and though in all cases a distinct reduction was obtained on testing for diastase, the amount present in some of the resting seeds was small. Slight differences in the amount of reduction are in part produced by extraneous factors, such as the strength and quantity of the solutions used, the fineness to which the seeds are ground, and the detailed treatment during extraction. These results do not coincide with those obtained by Acton,† who found that an extract from wheat grains which had been stacked for 28 years exercised no diastatic action on thin starch solution. He offers the suggestion that the diastase present in the freshly stored grains had been destroyed by oxidation, or by the influence of micro-organisms. Thus reference to the two first cases of oats cited in the tables shows that 10 grammes of fresh oats, with a germination capacity of 100 per cent., produced exactly the same reduction on testing as was produced by 10 grammes of oats from 8 to 10 years old, with a germination capacity of *nil*, under as precisely similar conditions as possible.

The least reduction was produced by maize, the strength of reduction in

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* Brocq-Rousseau and Gain ('Compt. Rend. Acad. Sci. Paris,' vol. 146, 1908, p. 545) state that peroxidase enzymes appear in seeds up to 20 years of age and may persist in some cases for 100 to 200 years.

† 'Annals of Botany,' vol. 7, No. 27, September, 1893.

Diastase Enzymes.

Kind of seed.	Age.	Reduction with Fehling's test.	Percentage of seeds germinated.	Percentage of seeds germinated with diastase.	Place of origin.
Wheat	21 years	Fairly strong	0	—	S. Australia.
"	20 "	Strong	0	—	"
"	19 "	Fairly strong	0	0 (unpricked) 0 pricked	"
"	18 "	Very strong	0	—	"
"	17 "	"	0	—	"
"	16 "	Strong	0	—	"
"	5 months	"	100	—	Victoria.

Diastase Enzymes.

Kind of seed.	Age.	Reduction with Fehling's test.	Percentage of seeds germinated.	Percentage of seeds germinated with diastase.	Place of origin.
Wheat	13 years	Strong	0	—	S. Australia.
"	12 "	"	2	—	"
"	11 "	"	12	—	"
"	10 "	"	28	24 (unpricked) 20 (pricked)	"
"	9 "	"	32	28 (unpricked)	"
"	8 "	"	32	25 "	"
"	7 "	"	62	—	"
Wheat (Steinwedel)	8 to 10 years	"	0	—	Victoria.
" (Duleith) ...	11½ years	"	0	—	"
Wheat (Indian King)	6 "	"	4	0 (unpricked)	"
Barley	5 months	"	100	—	"
" (Algerian)	8 to 10 years	"	0	0 (pricked) 0 (unpricked)	"
" (Chevalier)	8 to 10 years	Slight	0	—	"
Barley (Hallet Chevalier)	4 years	Very strong	54	51 (unpricked)	New South Wales.
Oats	1½ "	Slight	100	80 "	Victoria.
" (Golden)	8 to 10 years	"	0	0 (pricked) 0 (unpricked)	"
" (Algerian) ...	5 years	"	56	56 (unpricked)	"
"	3 "	Fairly strong	84	—	"
"	2 "	"	96	—	"
"	3 "	"	60	—	"
" (Calcutta) ...	4½ "	Slight	72	—	"
"	4½ "	Fairly strong	76	—	"
Rye	6 months	Strong	100	—	"
"	4½ years	"	32	32	"
"	8 to 10 years	"	0	—	"
Maize	6 months	Slight	100	—	"
Maize (Golden King)	4½ years	"	60	52	New South Wales.

the two specimens used being the same; but as material which had entirely lost its power of germination was not obtainable, the results in this case are not perhaps quite so convincing as in the case of the other cereals.

It might be urged that since the resting seeds were not stored in an absolutely dry condition, the persistence of the diastase might be due to its being reproduced as fast as it decomposed, but a comparison of the South Australian results with those from Victoria and New South Wales does not give any evidence of this.

2. *Proteolytic Enzymes.*—The first method adopted for the demonstration of these ferments was approximately the same as had been employed by Prof. Vines in his paper on "The Proteases of Plants,"* the difference being that in this case from none of the seeds had the integuments been removed before grinding.

The grain, 10 grammes in each case, was crushed by the hand mill, and put into 100 c.c. of distilled water and shaken for two hours. The material was then filtered and the filtrate was used as the digestive solution without precipitation of the enzymes.

Into bottles containing 50 c.c. of this solution were put 3 drops of hydrocyanic acid and 0·2 gramme of well-washed fibrin which had been carefully preserved in spirit. Into a similar bottle were put 50 c.c. of the same solution which was thoroughly boiled, and when cold 0·2 gramme of washed fibrin and 3 drops of HCN were added to this bottle also. These bottles were put into the oven at a temperature of 36° C. As in the case of diastase, control experiments in which commercial pepsin was employed were rendered impossible owing to the constant presence of traces of peptone mixed up with the pepsin, which was obtained both in the form of a powder, and as scales, but always containing the same impurities. Attempts to separate the pepsin and peptone by fractional dialysis failed.

After about 20 hours in the oven, application of the biuret test showed the presence of slight traces of peptone in the unboiled specimens, whilst the boiled specimens when similarly tested only gave the violet colour characteristic of undigested proteids.

On treating some of the aqueous solution from the seeds in exactly the same way as the above, with the single exception that no fibrin was added to the bottle, application of the biuret test showed the presence of minute quantities of peptone, thus indicating the occurrence of autolysis. The results obtained in this way are tabulated along with those obtained when dealing with solutions prepared by another method to be now described. In order to diminish autolysis and to obtain more satisfactory results, the enzymes were precipitated from their solutions. The method followed was almost identical with that described by Dean* when dealing with the proteolytic enzymes of *Cucurbita pepo.* Twenty grammes of the grain were ground and mixed with 100 c.c. of cold, boiled water, and shaken for two hours. The mixture was then filtered and the filtrate precipitated by a volume of saturated ammonium sulphate equal to that of the original filtrate. A white, more or less flocculent precipitate was thrown down, which was filtered off and dried overnight in an exhausted desiccator over strong H_2SO_4.

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* 'Annals of Botany,' vol. 20, 1906, p. 115.

The precipitate was scraped off by means of a sterilised knife as before, and dissolved in a small amount of cold, boiled water.

In testing for fibrin-digesting enzymes, 1 c.c. of the aqueous solution produced above was put into each of three sterilised test-tubes A, B, and C. The contents of the test-tube C were boiled well and allowed to cool. Into B and C were put 0·2 gramme of well-washed fibrin, and 5 drops of chloroform were added to each of the three test-tubes. The three test-tubes provided with corks were placed in the oven at 35° C. and left for an interval of time varying from two to four days.

Examination of the contents of all these test-tubes for the detection of peptone showed that B and C both gave faint biuret reaction, the boiled as well as the unboiled specimens.

A little of the liquid from each test-tube was examined under the microscope, with the result that it was found to be swarming with bacilli, by which the conversion of proteids into peptones had been wholly or partially effected.

This difficulty regarding the bacteria was due to the prolonged time necessary for the action of these ferments to become manifest; and it was evident that the addition of 5 drops of chloroform, and the subsequent plugging of the test-tubes, was inadequate to maintain antisepsis, so that it was necessary to adopt more stringent measures to destroy the bacteria, but still not to impede in any way the action of any digestive ferments which might be extracted from the resting seeds.

After many unsuccessful attempts to find some means of effectively fulfilling these conditions, the required end was attained by soaking the grain in strong chloroform for about five minutes after weighing it, and grinding it in the mill while still wet. Care was taken that all the bottles, apparatus, and water employed throughout the experiments were thoroughly sterilised beforehand. Prior to putting the test-tubes into the oven 5 drops of chloroform were added, and the tubes were stopped with plugs of cotton wool. In this way complete sterility was produced.

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* 'Botanical Gazette,' vol. 39, May, 1905, "Proteolytic Enzymes," p. 331.

The detailed results are enumerated in tabular form below, from which it will be seen that a proteid digesting enzyme is present, which is destroyed by moist heat at 100° C. As in the case of diastase, germination tests were performed in order to discover whether the germinating power was affected by the addition of commercial pepsin solution, or bore any relation to the persistence of the proteolytic enzymes in the resting seeds. Throughout the experiments the temperature of the oven was between 34° C. and 40° C.

Fibrin-digesting Proteolytic Ferments.

Kind of seed.	Age.	Method.	Time of digestion.	Reaction with biuret test.	Percentage germination.	Percentage germination with pepsin.	Place of origin.
Wheat	6 months	V.	50 hours	Faint	100	100	Victoria.
,,	21 years	V.	60 ,,	,,	0	—	S. Australia.
,,	20 ,,	V.	60 ,,	,,	0	—	,,
,,	6 ,,	D.	50 ,,	,,	0	—	,,
,, (Indian King)	6 ,,	D.	48 ,,	,,	8	4	Victoria.
Wheat	2½ ,,	D.	40 ,,	,,	68	68	S. Australia.
Barley	6 months	V.	60 ,,	,,	100	100	Victoria.
,, (Algerian)	8 to 10 years	V.	60 ,,	,,	0	0	,,
,, (Golden Drop)	8 to 10 years	V.	60 ,,	Very faint	14	8	,,
Barley (Hallet Chevalier)	4½ years	D.	48 ,,	Faint	54	25	New South Wales.
Barley	4½ ,,	D.	60 ,,	,,	100	—	Victoria.
Oats	5½ ,,	V.	60 ,,	Very faint	56	40	,,
,, (Golden)	8 to 10 years	V.	60 ,,	,,	0	0	,,
,,	1½ years	V.	60 ,,	,,	92	81	,,
,,	2½ ,,	D.	60 ,,	Faint	96	92	,,
,,	4½ ,,	D.	60 ,,	,,	62	58	,,
Rye	6 months	D.	64 ,,	Fairly good	100	96	,,
,,	4½ years	D.	64 ,,	,,	32	29	,,
,,	8 to 10 years	D. & V.	64 ,,	,,	0	0	,,
Maize	6 months	V.	70 ,,	Slight traces	100	—	,,
,, (Golden King)	4½ years	D. & V.	70 ,,	,,	60	60	New South Wales.

V. = Vine's method of preparing the proteolytic ferments, 'Annals of Botany,' No. 78, April, 1906, p. 115.
D. = Dean's method, 'Botanical Gazette,' vol. 39, May, 1905, p. 331.

Reference to the preceding tables shows that a fibrin-digesting enzyme is present in minute quantity in the resting grains of the cereals investigated, and that apparently its amount is not appreciably influenced by the age of the grains. Judging the amount of the ferment by its activity the amount present is small in all the seeds tested, the maximum activity being possessed by the rye seeds, and the minimum by the maize. The addition of commercial pepsin solution to the seeds does not in any case increase their percentage germination, and where the percentage germination is low tends to lower it still further.

Test for Erepsin.—Further investigations were carried on in order to find out whether erepsin ferments were present in the resting grains. The mode of precipitation of the enzyme has been previously described, the same method being employed as was used in testing for pepsin. Before grinding, the seeds were soaked for about five minutes in strong chloroform, as was done when testing for the existence of fibrin-digesting ferments in the seeds. One cubic centimetre of the aqueous solution of the precipitate produced by the saturated ammonium sulphate was put into each of three sterilised test-tubes A, B, and C. The same difficulties arose in the case of the samples of prepared pancreatin as were met with in the diastase and pepsin, the samples purchased giving the tryptophane reaction owing to the presence of amide impurities. As in the case of the fibrin-digesting enzyme, the contents of the test-tube C were boiled and allowed to cool. About 0·2 c.c. of Witte peptone was added to each of the tubes B and C; 5 drops of chloroform were dropped into each of the three test-tubes, and the mouths of the tubes were plugged with cotton wool. The three test-tubes were placed in the oven, which was kept at a temperature of 35° to 36° C. throughout the series of experiments. The test-tubes were left in the oven for about three days, during which time they were occasionally shaken at intervals.

The test adopted for the detection of products of erepsin digestion such as amides was the tryptophane test. About 4 or 5 drops of bromine water were added to each of the contents of the test-tubes A, B, and C, and without exception tryptophane was produced in the contents of the tube B, whilst no trace was observed in A or C.

The results demonstrate the occurrence of an erepsin ferment in the seeds, which is destroyed in water at 100° C. Experiments were also performed to determine the percentage of seeds capable of germination under the action of a solution of commercial pancreatin in water. The results of these experiments are stated in a special column of the tables below.

As in every instance the tryptophane reaction was well marked, in order to economise material the experiments for the detection of erepsin were limited to one sample of fresh and one of old grains.

The quantity of erepsin present must be fairly considerable as judged by the degree of activity of the extract.

Reference to the tables shows that no favourable effect on the germinative capacity of the seeds is noticeable as the result of soaking in weak pancreatin solution. The addition of a weak solution of pancreatin did not favour germination, but rather the reverse, except in the case of 4½-year-old rye. On repeating this latter test, however, the seeds only gave

Erepsin Ferments.

Kind of seeds.	Age.	Method.	Time acting on peptone.	Trypto-phane reaction.	Per-centage germina-tion.	Percentage germination with pancreatin.	Place of origin.
Wheat	2½ years	D.	65 hours	Good	100	98	S. Australia.
,, (Marshall's Prolific)	8 to 10 years	D.	65 ,,	,,	14	11	Victoria.
Barley (English)	4½ years	D.	65 ,,	,,	44	44	,,
,, (Algerian)	8 to 10 years	D.	65 ,,	,,	0	0	,,
Oats	1½ years	D.	65 ,,	,,	92	73	,,
,, (Golden)	8 to 10 years	D.	65 ,,	,,	0	0	,,
Rye	6 months	D.	65 ,,	,,	100	—	,,
,,	8 to 10 years	D.	65 ,,	,,	0	0	,,
,,	4½ years	D.	64 ,,	,,	34	30	,,
Maize	6 months	D.	65 ,,	Fairly good	100	—	,,
,, (Golden King)	4½ years	D.	65 ,,	,,	60	60	New South Wales.

D. = Dean's method of extracting erepsin, 'Botanical Gazette,' vol. 39, May, 1905, p. 331.

30 per cent. germination after treatment with pancreatin, so that the apparent rise was of accidental origin.

In concluding this section of the paper, the net results may be briefly summarised as follows :—

1. Although the germinating power continually decreases with advancing age, the enzymes persist comparatively unaffected.

2. Diastase is present in fairly large quantities in both fresh and old resting seeds of wheat, barley, oats, rye, and maize, being least active in the latter.

3. A fibrin-digesting ferment is present in traces in all the above-mentioned seeds.

4. Erepsin is present in considerable amount in all the above-mentioned seeds.

5. All these ferments are destroyed by being raised to the temperature of boiling water in the presence of moisture, but they are not destroyed by the immersion of the seeds for about five minutes in strong chloroform.

6. The maximum quantity of all the enzymes occurs in rye, and the minimum quantity in the maize.

7. The addition of dissolved ferments does not increase the percentage germination of old seeds, and where any effect at all is produced tends to lower it.

The Effect of Extremes of Temperature on the Germinating Power and Enzyme Contents of Seeds.

High Temperatures.—All the enzymes present in the resting grains are completely destroyed by moist heat at 100° C., and the same is true of the germinating power of the grains, no signs of germination being apparent in the seeds of cereals which have been immersed in boiling water for a few minutes. This appeared to suggest the possibility of some co-relation existing between the germinating power and enzyme reaction of seeds regarding their powers of resisting high temperatures, irrespective of the fact that no such co-relation exists between these two phenomena as regards their capacity for withstanding time.

To test whether any such connection really exists, many experiments were carried out, using dry instead of moist heat.

It is important that the seeds should be as nearly completely dry as possible, and for this reason before being used for an experiment they were taken from the store room, which was the driest place obtainable, and placed in sulphuric acid desiccators kept in an oven at about 35° C. for a week or more. The first series of experiments was carried out at 100° C., and the method adopted was as follows:—The dried grains were placed in perfectly dry test-tubes, which were fitted into holes in a sheet of cardboard. The test-tubes containing the seeds were placed in a vessel containing boiling salt solution, and the bulb of a thermometer was put into one of the test-tubes among the seeds. The sheet of cardboard prevented any steam reaching the open ends of the test-tubes, so that the grains were kept perfectly dry. The grains were kept at a temperature of 99° to 100° C., for different intervals of time varying from ¼ hour to 16 hours, and on removal from the test-tubes their germination capacity and enzyme reactions were investigated. The results of these investigations may be obtained in detail from the following tables.

The germinating power became gradually weakened as the interval of time during which the grains were subjected to this high temperature increased, but the same did not apply to the enzymic activity, for after 16 hours' exposure to 99° or 100° C. the actions of the enzymes were apparently in no wise impaired.

A different method had to be adopted in order to raise the temperature of the grains above 100° C. This was done by spreading the seeds, which had been previously dried in the desiccator, as before in a single layer, and placing them in an oven heated to the required temperature. The temperature of the oven was first raised to 120° C. and kept constant for an hour. The seeds thus treated were afterwards tested for their germinative capacity and their enzyme reactions, the former of which was found to be entirely lost in each kind of seed used, while the latter was still evident, though in certain cases it was markedly diminished.

The highest temperature at which the slightest possible traces of enzyme reactions remained visible was 130° C., when faint signs of saccharification of starch were produced by the diastase extracted from resting grains of barley subjected to this temperature. Throughout this series of experiments only fresh seeds were employed, whose germinating capacity before exposure to the high temperatures was approximately 100 per cent.

After the grains had been heated above 120° C. the nature of the precipitates was apparently changed. While the bulk of the precipitate was seemingly as copious or even more so than before the heating of the seeds, it was much more soluble in water than that obtained from the same material unheated, and the filtrate was thinner and less glutinous than before. This was especially pronounced in the rye, for the filtering process in this case lasted about two hours, while the same process with the fresh seeds which had not been exposed to high temperatures occupied as many days.

The tables appended below show the results of these experiments in detail, *i.e.* the effects produced in the seeds on exposure to abnormally high temperatures; the effects of extremely low temperatures on the seeds will be dealt with later.

The methods of precipitating both the diastase and the proteolytic enzymes were the same as those employed in the preceding section of the paper.

Briefly summarising the results set down in the tables, it is found that the most resistant of all the ferments to extremes of heat is the diastase of barley, which is not absolutely destroyed till the grains have been heated to 131° C. for an hour. The least resistant of the enzymes is apparently the fibrin-digesting enzyme, for it is destroyed entirely at 124° C. in every kind of seed tried. This result may, however, possibly be connected with the fact that the quantity of this ferment present even in the fresh grains is extremely small.

Whether the slight variation in the resistant power of the diastatic and proteolytic enzymes of different grains to dry heat indicates the existence of specific varieties of the different enzymes must remain for the present an open question, but in any case the most exact experiments merely indicate that the ferments in question are no longer capable of extraction and do not say whether they have been actually destroyed or merely coagulated and rendered insoluble. The coagulation temperature in the different seeds might

Temperature Extremes. High Temperatures.

Kind of seed.	Temperature.	Time seeds exposed.	Percentage germination.	Reduction with Fehling's test.	Biuret reaction.	Tryptophane reaction.
	° C.	hours.				
Wheat*	99—100	½	48	Strong	Faint	Good.
Barley	99—100	½	32	,,	,,	,,
Oats	99—100	½	48	,,	,,	,,
Wheat	99—100	1	24	,,	,,	,,
,,	99—100	4½	0	,,	,,	,,
Barley	99—100	4½	6	,,	,,	,,
Oats	99—100	4½	24	,,	,,	,,
Barley	99—100	6½	0	,,	,,	,,
Rye	99—100	6½	0	,,	,,	,,
Maize	99—100	6½	0	,,	,,	,,
Wheat	99—100	16	0	,,	,,	,,
Barley	99—100	16	0	,,	,,	,,
Oats	99—100	16	0	,,	,,	,,
Wheat	122	1	0	,,	Very faint	Fairly good.
Rye	122	1	0	Faint	,,	Good.
Maize	122	1	0	Faintest trace	,,	Very faint.
Wheat	124	1	0	Strong	None	Fairly good.
Rye	124	1	0	Faintest trace	,,	Faintest trace.
Maize	124	1	0	None	,,	None.
Oats	126	1	0	Faintest trace	,,	,,
Wheat	127	1	0	Faint	,,	Faint.
Oats	127	1	0	None	,,	None.
Wheat	128	1	0	Faintest trace	,,	Faintest trace.
,,	130	1	0	None	,,	None.
Barley	130	1	0	Faintest trace	,,	,,
Oats	130	1	0	None	,,	,,
Barley	130	1	0	,,	,,	,,

* [In my paper on the vitality of seeds, wheat and barley are given as withstanding a day's dry heat at 100° C. The error is due to the transcription of 1 h. into 1 d., and the records are for one hour's heating and not one day's, the somewhat higher percentages being possibly due to more perfect drying.—Alfred J. Ewart.]

naturally vary somewhat, since their structure, composition, and power of retaining moisture all vary to a certain extent.

The diastase and the erepsin of the resting seeds appear to be almost equally resistant to dry heat, or at least there is more variation between the diastases of different resting seeds than between the diastase and erepsin of the same seed.

Above 100° C. no seeds of any kind were found to be capable of germination, and the germinating power was absolutely lost in those seeds which had been subjected in a dry condition to a temperature of 99° to 100° C. for 5½ hours. Just* showed that as seeds are dried their resistance to dry heat increases, and von Hohnel† found that many fully dried seeds could withstand an hour's exposure at 110° C.

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* 'Cohn's Beiträge,' vol. 2, 1877.

† 'Haberlandt's Wiss.-prakt. Unters.,' vol. 2, 1877.

Low Temperatures.—The exposure of the grains to low temperatures, both in the dry and moist conditions, had different effects from their exposure to high temperatures. The mode of carrying out these investigations was as follows: Seeds of fresh wheat, barley, oats, rye, and maize were dried in the same manner as when testing for the effects produced by abnormally high temperatures, and placed in perfectly dry glass tubes, which were carefully sealed off, but which previously to sealing had been weighted with shot.

Samples of the same kinds of seeds were put together with shot into loosely woven muslin bags, and the tubes and bags were lowered into a flask of liquid air. The weighting of the tubes and bags was necessary owing to the specific gravity of the liquid air being about equal to that of water.

The liquid air remained in the flask for about three days and all the seeds were completely immersed in it for fully two days.

The seeds were removed from the liquid air and some of each kind were set for germination, while corresponding seeds from the same packets which had not been subjected to the temperature of the liquid air were also set to serve as controls. Also some of the seeds from the tubes and muslin bags were ground up and their ferments precipitated as before. Neither the germinating power nor the enzyme reactions appeared to be appreciably affected in the case of any of the cereals by the exposure to the extreme cold of the liquid air, the temperature of which is approximately −200° C. No constant difference was noticeable between the effects of exposure in sealed tubes and of exposure in muslin bags where the seeds were in direct contact with the liquid air.

The slight drop in the percentage germination after exposure to liquid air in sealed tubes in the case of barley and rye, and in the case of wheat, oats, and rye where the seeds were in direct contact with the liquid air, is probably the result of these samples containing a few seeds whose power of germination was at a low ebb. In any case the differences are very small, and would be almost within the limit of error, were they not all on the same side.

As no means were available of obtaining lower temperatures, it was impossible to arrive at the satisfaction of destroying the ferments by abnormally low temperatures, if this be possible. Somewhat similar sets of experiments were performed by Brown and Escombe,* who kept various kinds of seeds exposed to liquid air enclosed in vacuum-jacketed tubes for 110 hours, and then slowly thawed them. They proceeded to test the germinating power of these seeds together with control specimens, but did not discover any appreciable difference between that of the seeds which had been exposed to liquid air and the control specimens which had not been so exposed. The same conclusions were arrived at by Thiselton-Dyer,* who subjected seeds to a temperature of −250° C. for a shorter period. Becquerel† also performed experiments dealing with this subject.

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* 'Science,' N. Ser., vol. 8, 1898, p. 215.

The results of the experiments performed are as follows :—

Temperature Extremes. Low Temperatures.

Kind of seed.	Percentage germination (normal).	Percentage germination after liquid air.	Reduction with Fehling's test.	Biuret reaction after fibrin digestion.	Tryptophane reaction after Witte-peptone digestion.	Contained in—
Wheat ……	100	100	Strong	Faint	Good	Sealed tube.
Barley ……	100	96	”	”	”	”
Oats ………	92	92	”	”	”	”
Rye ………	96	90	”	”	”	”
Maize ……	90	90	”	”	”	”
Wheat ……	100	96	”	”	”	Muslin bag.
Barley ……	100	100	”	”	”	”
Oats ………	92	90	”	”	”	”
Rye ………	96	92	”	”	”	”
Maize ……	90	90	”	”	”	”

As regards the ferments, there was not the faintest perceptible difference between those precipitated from the two sets of seeds, although it must be remembered that a difference will only be perceptible when a relatively large part of the original amount of ferment has been destroyed or rendered inactive or insoluble.

It is of great interest to note that the enzymes present within the resting grains of the five different genera of cereals employed throughout these experiments are not destroyed when the thoroughly dried seeds are subjected to the extraordinarily wide range of temperature of −200° C. to +120° C., *i.e.* a range of 320° C.

The enzymes of a few varieties of seeds such as the diastatic ferment of barley retains a certain amount of its activity when the range of temperature through which the seeds have been exposed is −200° C. to 130° C., *i.e.* a range of 330° C.

The range of temperature through which the capacity for germination is retained is from −200° C. to 100° C., *i.e.* a range of 300° C., above this the power is apparently entirely lost.

The conclusions arrived at in this section serve to substantially verify that drawn from the last section, that the capacity for germination is not dependent upon the existence of enzymes in the resting seeds of the cereals mentioned, although the question will not be absolutely closed until it is found possible to germinate seeds which contain no enzymes in the resting condition, or in which these enzymes have been destroyed. Since enzymes appear to retain their activity within a wider range of conditions than does the capacity for germination, this is likely to be a matter of the utmost difficulty, or may be impossible.

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* 'Roy. Soc. Proc.,' vol. 65, p. 362, 1899.

† 'Ann. Sci. Nat., Bot.,' ser. 9, vol. 5, 1907.

Before concluding the series of experiments in connection with this section of the paper the resistance to extreme cold of certain other varieties of seeds was also tested. Some of the different kinds of seeds were tied up in loosely woven muslin bags, together with shot to ensure their sinking when immersed in liquid air. The bags were lowered into a flask of liquid air in which they were left for one and a half days.

One hundred of each kind of seeds from the liquid air were set to germinate on damp blotting paper in a special germinating box, whilst 100 of each kind which had not been exposed to the extreme temperature of liquid air were set to germinate on damp blotting paper alongside them. The varieties of seeds employed were chosen from sorts possessing widely differing characters, including some which, being sensitive to desiccation, might also be sensitive to extreme cold.

The names of the seeds used are enumerated in the tables, accompanied by the relative numbers which germinated under normal conditions and after exposure to liquid air respectively. The third column contains data supplied by Prof. Ewart for comparison between the resistance to extreme cold and to desiccation.

Reference to the table shows that in not a single instance were the seeds entirely killed as the effect of their immersion in liquid air.

The influence of the low temperature is naturally most pronounced in the case of samples with a comparatively low germination capacity in which a number of the seeds are only just able to germinate under the most favourable conditions.

In the case of the carrot seed, freezing appeared to increase the percentage germination, but on re-testing the original seeds a percentage germination of 65 was obtained; possibly the first test was discontinued too soon.

The liquid air apparently exerts a retarding influence on the germination as, except in the isolated case of the cress seeds, in which signs of germination were apparent in 100 per cent. of both sets of seeds one day after sowing, germination was always noticeable in the seeds grown under ordinary conditions before those which had been subjected to the intense cold of liquid air.

Germination Tables.

Kind of seed.	Percentage germination. (Normal.)	Liquid air.	Resistance to desiccation.
Apple (*Pyrus malus*)	12	4	Sensitive to severe desiccation.
Turnip (*Brassica campestris*)	91	88	43 per cent. after 42 days' desiccation at 37° C.
Cress (*Lepidium sativum*) ...	100	100	30 per cent. after 4 weeks in absolute alcohol.
Carrot (*Daucus carota*)	36 to 65	59	Lasts 10 years in dry air.
Haricot (*Phaseolus multiflorus*)	100	90	2 per cent. after 45 days' desiccation at 37° C.
Hemp (*Cannabis sativa*)......	24	7	Nil after 15 days' desiccation at 37° C.
Mustard (white) (*Brassica alba*)	85	72	Lasts 10 years in dry air.
Lobelia erinus	51	13	Sensitive to prolonged extreme cold (De Candolle).
Parsnips (*Peucedanum sativum*)	45	18	Sensitive to extreme desiccation.
Parsley	28	1	" " "
Pea (*Pisum sativum*)	95	75	Nil after 42 days' desiccation at 37° C.
Radish (*Raphanus sativus*)...	97	88	Lasts 10 years in dry air.
Ricinus cambogiensis	100	100	*R. communis* 40 per cent. after 28 days' desiccation.
Sunflower (*Helianthus annuus*)	70	65	51 per cent. after 42 days' desiccation at 37° C.

The fact that in every experiment except two there is a lower percentage germination in the severely frozen seeds, and that in no instance is the reverse the case, signifies that to some extent freezing in the liquid air is deleterious to the germinative power of seed. Another noteworthy observation is that there does not appear to be any particular class of seed which is more injured by the extreme cold than any other class, *e.g.*, of three kinds of oily seeds tested, viz., Hemp, Helianthus, and Ricinus, while the first was strongly affected, the second was little, and the last-named seed not at all injured by −200° C. for two days.

The starchy seeds of cereals are, however, as resistant to the effects of exposure to liquid air as are the oily seeds.

Lobelia erinus seeds were selected as good subjects for experiment on the strength of the statement of De Candolle* that dry seeds of *Lobelia erinus* lose their vitality sooner at very low temperatures than at ordinary ones.

The results tabulated in this paper show that the vitality of some is lost, but as 13 per cent. were found to germinate after exposure for one and a half to two days to a temperature of approximately −200° C., it is probable that if the time of their exposure were increased somewhat the vitality of all the seeds would be destroyed.

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* Pfeffer, 'Physiology of Plants,' Engl. translation, vol. 2, p. 234.

From the third column on the list it can be seen that, on the whole, though not without exception, the resistances to extreme cold and to extreme desiccation are approximately parallel.

4. *The Respiratory Activity of Resting Seeds.*

In this series of experiments the respiration of certain other characteristic kinds of seeds was tested in addition to the foregoing cereals.

Whether dried seeds respire at all, and if they do to what extent, is one of the most discussed problems in plant physiology, especially in connection with the views as to whether the life in resting seeds is merely at a low ebb or is entirely suppressed.

The apparatus employed was Aubert's improved form of that of Bonnier and Mangin, and, as was stated in a previous paper dealing with the respiration of gynæcia,* the machine gave complete satisfaction, provided that certain precautions were taken. Before each set of experiments the mercury was removed from the apparatus and was thoroughly cleansed by several washings in strong hydrochloric acid, followed by several washings in distilled water, and then being passed through a filter to dry.

This precaution was found to be of extreme importance, for in the presence of any impurities such as zinc in the mercury, the inlet of a sample of air into the tube produced oxidation of the zinc, and a consequent diminution of the volume of the sample of air when allowed to stand in the apparatus for a short time.

The NaOH used was a 40-per-cent. solution, and the pyrogallic acid was a saturated solution diluted to one-fourth its original strength.

The seeds employed were the ordinary cereals, and also *Eucalyptus globulus*, *Acacia melanoxylon*, *Cytisus laburnum*, *Setaria italica*, *Ricinus cambogiensis*, *Cannabis sativa*, and *Pinus insignis*—and the experiments were performed in four series.

1. The seeds used were tested as received from storage.
2. The seeds before being tested for their respiratory activity were dried in the oven for eight days at a constant temperature of 45° C.
3. Samples of the seeds after drying for eight days at 45° C. were further heated in the oven for three days. During the daytime the temperature of the oven was 100° C., whilst at night the temperature fell to about 70° C.
4. Some of the above seeds were still further heated to about 130° C., when all were killed, and the gaseous exchanges were again tested.

For each respiratory test a weighed quantity of each seed was passed up into the upper part of a narrow, perfectly dry test-tube, containing a known volume of air, over mercury. These test-tubes had been previously sterilised by dry heat in all cases, although with the thoroughly desiccated seeds this precaution is not really necessary, except as a means of drying the tube.

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* 'Annals of Botany,' 1908.

The tubes containing the seeds and mercury were set up vertically in a shallow dish of mercury, where they were kept for from 5 to 15 days. After this time had elapsed, samples of the contained air were drawn into the Bonnier and Mangin apparatus, in which their composition was ascertained.

Those seeds which were found to emit no carbon dioxide in their ordinary stored condition were not further tested for signs of respiration in the more completely desiccated state, but the quantity of moisture was ascertained in every kind of seed used. The relative amounts of water present in the seeds at different stages of desiccation are set down in a special table which follows the respiration tables given below. Throughout these experiments the seeds used were the freshest obtainable, and several analyses were made of each sample of air, the results tabulated below being the mean of these analyses.

Respiration of Seeds.

Kind of seed.	State.	Weight.	Volume of air.	Time.	Mgrms. CO_2 per grm. of seeds per day.	Percentage volume of O_2 absorbed per day.
		grms.	c.c.	days		
Acacia melanoxylon ………	As stored	2	5	6	0·005	0·15
Avena sativa ………	„	6	18	7	0·0	0·07
Cannabis sativa ………	„	5	4	6	0·0	0·3
Cytisus laburnum ………	„	5	4	6	0·001	0·15
Eucalyptus globulus ………	„	2	2	6	0·0	0·05
Hordeum sativum (barley)	„	6	18	7	0·0	0·014
Setaria italica ………	„	2	5	6	0·001	0·03
Pinus insignis ………	„	5	5	6	0·0006	0·13
Ricinus cambogiensis ………	„	5	7	6	0·0	0·10
Secale cereale ………	„	6	15	6	0·004	0·15
Triticum vulgare ………	„	6	18	5	0·13	2·5
Zea mais ………	„	6	15	5	0·004	0·02
Acacia melanoxylon ………	Dried at 45° C. in dry heat for 8 days	2·0	8	5	0·0	0·14
Setaria italica ………	„	1·2	6·5	4	0·0	0·02
Pinus insignis ………	„	1·6	5·5	4	0·0	0·06
Secale cereale ………	„	2·0	6	5	0·0	0·02
Triticum vulgare ………	„	2·3	6	5	0·0	0·00
Zea mais ………	„	2·0	5·5	5	0·001	0·12
„ ………	Further dried for 3 days at 100° C., dry heat	2·0	5·5	5	0·001	0·05

Table of Moisture contained in the Seeds.

Kind of seed.	Weight of seeds as stored.	Weight of seeds after 7 days at 45° C.	Weight of seeds after further heating to 100° C. for 3 days.	Percentage weight of moisture lost after 7 days at 45° C.	Percentage weight of moisture lost after 3 days at 100° C.	Respiratory activity of stored seeds.	Respiratory activity of seeds after 7 days at 45° C.	Respiratory activity of seeds after further heating to 100° C. for 3 days.
	grammes.	grammes.	grammes.					
Acacia melanoxylon	2 ·43	2 ·33	2 ·32	3 ·29	4 ·5	Slight	Nil	Nil.
Avena sativa	5 ·0	4 ·59	4 ·48	8 ·20	10 ·4	Nil	"	"
Cannabis sativus	2 ·37	2 ·27	2 ·21	4 ·20	6 ·8	"	"	"
Cytisus laburnum	2 ·47	2 ·25	1 ·68	8 ·80	31 ·9	Very slight	"	"
Eucalyptus globulus	0 ·44	0 ·38	0 ·38	13 ·60	13 ·60	Nil	"	"
Hordeum sativum	5 ·0	4 ·70	4 ·52	6 ·0	9 ·6	"	"	"
Setaria italica	2 ·41	2 ·24	2 ·18	7 ·0	9 ·5	Very slight	"	"
Pinus insignis	3 ·26	3 ·10	3 ·09	4 ·9	4 ·9	Extremely slight	"	"
Ricinus cambogiensis	5 ·05	4 ·89	4 ·72	3 ·2	6 ·5	Nil	"	"
Secale cereale	2 ·97	2 ·73	2 ·62	8 ·0	11 ·9	"	"	"
Triticum vulgare	2 ·48	2 ·28	2 ·19	8 ·0	11 ·6	Strong	"	"
Zea mais	2 ·39	2 ·20	2 ·10	7 ·9	12 ·1	Slight	Trace	Trace.

Comparing the preceding tables it is seen that a feeble respiratory activity is shown by some of the seeds examined in the ordinary stored condition, which varies according to the seed and to the amount of moisture it contains. Oats, hemp, barley, Eucalyptus, and Ricinus showed no evolution of carbon dioxide, and the trace of oxygen absorbed may be the result of chemical oxidation or physical absorption.

Respiration was surprisingly active in the fresh wheat as obtained from the seedsman, although not more than 1/300 part of what it is in an active seedling. Further, the evolution of carbon dioxide ceased after a comparatively small degree of desiccation. Reference to the moisture tables shows that the percentage of moisture contained by the wheat was not relatively large as compared with the other seeds employed.

As might be expected from the fact that the seeds of *Acacia melanoxylon* are completely covered by a cuticle, which in the fully dried seed can withstand nearly 45 minutes' immersion in strong sulphuric acid without becoming permeable to water, the percentage of moisture eliminated by slow desiccation in dry heat is less marked than in any of the other varieties of seeds. The carbon dioxide evolved from the air dried seeds does not amount to 1/10000 of the amount evolved from an active seedling, and is, in fact, nearly within the limit of error, and may possibly be the result of oxidations taking place in the arillar appendages of the seeds.

In any case, all respiratory activity as evidenced by the gaseous exchanges completely ceased after the same seeds had been desiccated at 45° C. for seven or eight days. For purposes of control various dead seeds, fragments of dead wood, etc., were similarly tested in the air dry condition. In no case could any sign of an evolution of carbon dioxide be detected, and the fact that in some cases a trace of oxygen disappeared is not surprising, considering the structure of the materials tested, and their large bulk relatively to the amount of the enclosed air.

The above results indicate that respiration is not a function of completely dry seeds, nor even of seeds after a mild degree of drying, for only in one isolated instance, that of *Zea mais,* was there the faintest trace of any apparent respiratory activity present after remaining at 45° C. for one week. The amount of carbon dioxide produced in this case was less than one-millionth of that produced by an active seedling in the same time, and was evidently the result of the slow outward diffusion from the bulky seeds of carbon dioxide formed while in the air dry condition. The same applies to the traces of oxygen absorbed, as the gaseous relationships inside and outside the seed become equalised.

This is made even more evident by the fact that the greater number of the fresh seeds in the air dry condition as obtained from the seedsman exhibited no respiratory activity whatever, although they contained quite appreciable quantities of water. Among these non-respiring seeds *Ricinus* is included, though in another species of *Ricinus*, Becquerel* states that an active interchange of gases does occur in the dried seeds, which he asserts to be a purely non-vital chemical action.

The experiments of Kolkwitz† carried out on barley illustrate the important effects produced on the respiration of seeds by the presence of moisture in the seeds. He found that—

1 kilogramme barley grains at summer temperature gave off 3·59 m. mg. of CO_2 in 24 hours when 19 to 20 per cent. of water was present;

1·4 m. mg. with 14 to 15 per cent. of water; and

0·35 m. mg. with 10 to 12 per cent. of water.

Becquerel‡ also discusses the effect of the presence of moisture in the seeds on the respiratory activity of the seeds in his extensive researches on the latent life of seeds, a short account of which also appears in the 'Comptes Rendus,' vol. 143, No. 26, December 24, 1906, p. 1177.

Summary.

The resting seeds of cereals such as wheat, maize, barley, oats, and rye all contain diastatic, fibrin-digesting, and ereptic ferments in appreciable amount. These ferments retain their activity without appreciable change in stored dry seeds for 20 or more years, that is long after the power of germination has been lost, which takes place in wheat after 11 to 16 years, barley 8 to 10 years, oats 5 to 9 years, maize and rye over 5 years. The life of the stored seeds is largely dependent upon the climatic conditions, a dry climate favouring longevity. Thus South Australian wheat lasts longer than that stored in Victoria, and still longer than that obtained from New South Wales. The difference is, however, not shown strongly until after the fourth or fifth year, South Australian wheat being still one-third germinable after 9 to 10 years, whereas wheat stored in Victoria had entirely lost its vitality by this time.

No relation was noted between the vitality of seeds and the persistence of enzymes in them, but since the enzymes persisted longer than the power of germination, the question as to whether germination could take place in the absence of any pre-existent enzymes remains to be answered. In any case

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* 'Comptes Rendus,' vol. 143, 1906, p. 974.

† 'Ber. d. Bot. Gesell.,' vol. 19, p. 285, 1901.

‡ 'Ann. Sci. Nat., Bot.,' ser. 9, vol. 5, 1907.

no otherwise non-germinable seeds could be excited to germination by the addition of any kind of enzyme, and where the germination was feeble the addition of enzymes usually lowered the percentage germination and often delayed germination also to some extent.

The erepsin appears to be more abundant than the pepsin, but otherwise in the cases of all three ferments greater differences are shown between different samples of the same age than between different seeds, or between the same seeds of varying ages. Pepsin appears, however, to be more abundant in rye than in any other cereal, and is almost absent from maize. Dry oats, barley, and wheat can in part resist a temperature of 99° to 100° C. for 1 to 4½ hours; after 6 hours' exposure all are killed, but the ferments are apparently unaffected. All the ferments are destroyed after an hour's dry heat at 130° to 131° C. The pepsin appeared to be least (1 hour at 124° C.), the erepsin more (1 hour at 124° to 128° C.), and the diastase, especially of barley, most resistant to dry heat (1 hour at 124° to 131° C.).

Two days' exposure to liquid air, although it delays the subsequent germination and may also decrease the percentage, does not absolutely destroy any of the seeds tested and does not appreciably affect the ferments in any of the cereals. The dry diastase of barley is therefore able to withstand a range of temperature of $-200°$ to $+130°$ C. It is therefore thermally a highly stable chemical compound.

Many seeds, including all cereals, give off appreciable quantities of carbon dioxide when stored in the air dried condition, but others show no signs of respiration whatever. The respiration of air dried wheat is especially pronounced, but in practically all cases every sign of respiration ceases when the seeds are moderately desiccated, although in the case of large seeds like maize minute traces of carbon dioxide may continue to escape for a time.

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CROONIAN LECTURE.—*The Functions of the Pituitary Body.*

By E. A. SCHÄFER, F.R.S.

(Lecture delivered June 10,—MS. received July 22, 1909.)

The observation of P. Marie (1885) that the disease to which he has given the name "acromegaly" is associated with tumours of the pituitary body has caused this organ to attract the recent attention of pathologists to a greater degree than any other of the structures which were formerly classed together under the generic name "ductless glands." Since Marie's description of that disease, very many cases have been recorded, and in most of these the same association has been noticed.

The most striking sign of acromegaly is the increased growth of certain parts of the skeleton, especially the lower jaw and the extremities of the limbs, with hypertrophy of the connective tissue; indeed, the enlargement of the hands and feet is frequently the first change which calls attention to the existence of the disease, the patient finding that his gloves and boots are becoming too small for him. In the later stages there is dorsal kypho-scoliosis. Headache is a prominent symptom, polyuria is often present, and the eyesight is frequently affected. Acromegaly usually occurs in adults, often about middle age, although it may begin during adolescence. An allied affection—(pathological) gigantism—which occurs before normal growth is completed, is accompanied, in addition to many of the above symptoms, by an increase in length both of the limb bones and of the trunk, so that the patient affected attains an altogether unusual stature. This also has been found in most cases that have been examined to be associated with tumours of the pituitary body and a concomitant enlargement of the sella turcica. It is probably the case that the changes in the skeleton in acromegaly and gigantism are due to the same cause, operating, perhaps, at different stages during the progress of growth, and that this cause is to be found in an alteration in the functions of the pituitary body.* Assuming from what is known of the above diseases that the functions of this organ, or of a part of it, have to do with the growth and nutrition of the skeletal tissues, it has still to be decided whether the increased and abnormal growth which is met with in them is due to diminution or excess of the activity of the gland. The former view was taken by Marie, who noticed—as others have done since—that the nature of the tumour which is found after death is often such as to have produced complete destruction of the organ, a cancerous or sarcomatous formation having been frequently described. Those who uphold this view look upon the gland as in some way—probably by means of an internal secretion or hormone—regulating the growth of the skeleton, which in the absence of such regulation proceeds abnormally. But the opposite view (Tamburini, 1894, and Woods-Hutchinson, 1894) has also been advocated, viz., that the symptoms of acromegaly and of gigantism are due to a hypertrophic condition of the pituitary, or, according to the very probable suggestion of Woods-Hutchinson, of its anterior lobe alone, which may be considered to produce too great a quantity of a hormone which stimulates bone-growth. The most important argument in favour of this view is derived from the fact that the pituitary tumours which have been found to be associated with the acromegalic condition have in many instances, especially where the tumour has not been unusually large, been described as a simple glandular hyperplasia of the anterior lobe. And in a few cases of acromegaly which have been noted to be unaccompanied by any distinct enlargement of the pituitary, the glandular cells have been described as unusually full of the granules which are generally regarded as indicative of the secretory activity of the cells.

On the other hand, the numerous instances in which after death the glandular substance of the organ has been found entirely destroyed and replaced by cells of the types met with in malignant growths seem at first to offer difficulty to the acceptance of this view, and to favour the opinion propounded by Marie that the symptoms have been produced by suppression of the internal secretion. But to this it may be replied that in such cases—as, in fact, is not unfrequent in tumours of glandular organs—it is possible to assume that the tumour at its beginning was non-malignant and of the nature of a simple hyperplasia, the malignancy of character being established later, and only then proceeding to destruction of the glandular type of cell.

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* *Cf.* Woods-Hutchinson (1898, 1900). According to Woods-Hutchinson, the connection between acromegaly and gigantism was first suggested by Cunningham (1891).

The chronic character of the affection favours on the whole this supposition. For it appears to be established experimentally that complete extirpation or destruction of the gland is incompatible with continuance of life for more than a few days at the utmost. If we assume—which we are not entirely justified in doing—that destruction by disease will have a similar result, then we should expect, if acromegaly be due to hypertrophy and increase of activity of the gland, that as long as such a tumour is merely glandular and benign, the series of symptoms which characterise the disease would gradually develop, and it is a known fact that in most recorded cases it has pursued a slow course with a gradual development of the characteristic signs. It is unlikely that at this stage the tumour which is forming is already malignant, and especially that it has assumed a sarcomatous character, which is that which has perhaps been most often described *post mortem* in this disease. It is more probable that the malignant character has become developed shortly before death, and by no means improbable that death has resulted from entire suppression of the function of the gland owing to destruction of the normal glandular cells by those of a malignant nature. One must at the same time bear in mind the existence of other possible causes or contributory causes of death, such as the pressure of the tumour upon the base of the brain and the mere existence of malignant disease. But in many cases the tumour has not been of sufficient size to justify death being attributed to these causes, and the assumption that it has resulted from destruction of the normal glandular tissue is probably correct.

If the symptoms of acromegaly are due to an excess of secretion from the gland, one would not expect amelioration as the result of pituitary feeding. Campbell Geddes (1908) mentions a case in which the patient became rapidly worse when pituitary and ovarian extracts were given. In this case the pituitary was 30 times the normal weight, and showed simple hypertrophy of the anterior lobe tissue only. There was no polyuria, although this condition is often found either with or without glycosuria in both acromegaly and gigantism. Its occurrence is best explained, as will be presently seen, by supposing the posterior lobe, or, at least, the pars intermedia, to participate in the hyperfunctioning of the anterior lobe.

Before proceeding further it is necessary to refer to certain facts relative to the structure, development, and functions of this organ.

Development.

Regarding its development, it is known to have a double source of formation, a hollow extension from the buccal ectoderm towards the base of the brain being met by a hollow extension of the neural ectoderm occupying the situation of the future infundibulum. The two extensions eventually grow together and constitute the pituitary body, the buccal ectoderm, which loses its connection with the alimentary tube, forming the anterior lobe and pars intermedia, *i.e.* by far the larger portion of the organ; while the neural ectoderm becomes developed into the posterior or nervous lobe. This retains in some animals its hollow connection through the infundibulum with the third ventricle of the brain, although in man and other Primates it becomes entirely solid.*

Structure.

The structure of the parts which are thus so differently formed in the embryo is also, in the adult, entirely different. For while the pars nervosa s. posterior shows no development of any tissue which can be supposed to possess either nervous or glandular activity—consisting as it does mainly of neuroglia elements with very few vessels—the pars anterior is formed of a highly differentiated epithelium-like tissue, very richly supplied with large and thin-walled capillary blood-vessels, many of its cells being filled with granules such as are characteristic of glandular structures. The appearance of the pars anterior is, in fact, precisely that of an organ which has the property of forming a secretion which is passed from its cells directly into the blood-vessels, and one would, without hesitation, class it amongst the internally secreting glands.

The pars anterior in man and in most animals is separated from the posterior lobe by a cleft-like cavity, which is the remains of the original hollow of the outgrowth from the buccal ectoderm. But the epithelial tissue immediately adjoining this cleft, and especially that which impinges on the pars nervosa, is of a different character from that of the pars anterior. The cells are less distinctly granular: they tend to be arranged in islets separated by intervening tissue which is continued between them from the pars nervosa, and many of the islets are hollow, forming small vesicles which are occupied by a "colloid" material. The inter-epithelial tissue is far less vascular than that of the pars anterior. But this tissue also exhibits "colloid," which is contained in spaces prolonged into the pars nervosa, and the same material can even be seen discharging into the prolongation of the infundibulum which enters the pars nervosa. Indeed, in some animals (*e.g.* cat) the infundibulum extends as a hollow canal as far as the pars intermedia, and this canal receives the colloid secretion of that part of the gland. The pars intermedia differs, therefore, from the pars anterior not only in the structure of its cells but also in the fact that its secretion—which is no doubt represented by the "colloid" material—is, in all probability, not taken up directly into the blood but is passed into the infundibulum and thus into the third ventricle.

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* This description of the development and structure of the mammalian pituitary body is based upon the investigations of Herring (1908), which is itself supplementary to and in many particulars confirmatory of the results of former observers.

The discovery of this difference, which must be regarded as a fact of great importance in the physiology of the pituitary, is due to the investigations of Herring (1908), who has further found that the amount of such colloid which is discharged into the infundibulum is greatly increased after removal of the thyroid in animals. It is true that an increase in the amount of "colloid" in the pituitary body had previously been noted after thyroidectomy; but this "colloid" was located by previous observers in the anterior lobe, and was supposed to pass into the blood-vessels, whereas it has been shown by Herring to be a product of the cells of the pars intermedia and to pass into the infundibulum and third ventricle.

Functions.

The first investigations of a strictly physiological character which were instituted to determine whether the pituitary body possesses any active function were those of Oliver and myself (1895). We found that aqueous or saline extracts—which may be boiled without losing their activity—produce, when injected into the blood-vessels, a rise of blood-pressure which is comparable to that produced by similar extracts of the suprarenal capsules. We further showed that this effect is produced by an action upon the peripheral arteries, which are caused strongly to contract, in this also resembling the action of the active principle obtained from the suprarenals; but far more prolonged, and not due to the presence of that substance in the extract. We did not in these experiments obtain any marked effect upon the rate of the heart's beat, an acceleration of which is a characteristic feature of the action of suprarenal extract, after the vagi have been cut or paralysed (prior to which there may be some inhibition). Nor did we differentiate between the action of the different parts of the gland, having used extracts of the whole pituitary body.

Howell (1898) carried the investigation further, and added considerably to our knowledge of the action of extracts of the gland. He split it into anterior and posterior parts, and determined that whilst the extract of the former is without physiological activity when injected into a vein, that of the latter produces the effects upon blood-pressure and blood-vessels which Oliver and I had obtained from extracts of the whole gland. Howell further found the rise of blood-pressure to be accompanied by a slowing in the action of the heart, and that both the raised blood-pressure and slow cardiac rhythm might be maintained for a considerable time. And that if a second dose be administered intravenously within a certain time—which varies from half an hour to an hour or more—after the first dose, these effects are not repeated—in other words, a certain immunity is established which only slowly passes off.

Swale Vincent and I (1899) repeated Howell's observations. We were able to confirm them in almost every particular, but found that the cardiac slowing described by Howell is not constant, and that when present it is not abolished by section of the vagi or the action of atropine. It is, therefore, of peripheral origin, and not due to the same cause as the inhibition which often accompanies the action of adrenin, which is brought about by an action upon the cardio-inhibitory mechanism in the bulb. We also found that not only is there generally no *rise* of blood-pressure resulting from a second or third dose of the extract of posterior lobe, but there is invariably a *fall*, which, however, lasts only a short time. We showed that this fall of blood-pressure is due to a depressor substance acting upon the blood-vessels; that the substance is soluble in alcohol, in which the pressor substance is insoluble; and that it is not identical with cholin, which has a similar action, and might be supposed to have been extracted from the nervous tissue of the lobe. These facts have now been corroborated by many experimentalists, and it has recently been shown that they hold good for extracts of the human pituitary (Halliburton, Candler, and Sikes, 1909).

But the action upon the circulatory organs does not exhaust the effects of such extracts. For in the course of certain experiments which Dr. R. Magnus and I were conducting in the summer of 1901, we incidentally noticed, as one of the results of intravenous injection, a marked increase in the flow of urine from the ureters. Pursuing the subject further, in association with Herring (1906), the fact became evident that the aqueous extract of the posterior lobe—including the pars intermedia, which comes away with it when it is separated from the anterior lobe—has a specific action both upon the renal vessels and upon the kidney cells. For whereas this extract produces contraction of most of the arteries in the body, it has the opposite effect upon those of the kidney, causing them to dilate, although this dilatation, which is very marked and lasting, may be preceded by a short period of contraction. The increase in flow of urine, although no doubt greatly assisted by the dilatation of the kidney vessels, which is coincident with a rise in general blood-pressure caused by contraction of other arteries, is not entirely produced by the vascular changes. For it may occur without them, as in the case when a repeated dose of the extract is administered intravenously within a short interval. In such cases, as we have seen, the rise of blood-pressure may fail altogether, or even be replaced by a temporary fall, and there may also be no further dilatation of the kidney produced; nevertheless, the diuretic effect may still occur, and this can only be explained by supposing that there is some substance in the extract which acts by directly stimulating the secretory activity of the cells. Moreover, I have had occasion to observe that the converse of this experiment may occasionally be obtained, and this with a first dose; the normal effects of rise of blood-pressure and dilatation of kidney being produced without any increase in flow of urine (see fig. 1).

It is further noticeable that in a large proportion of experiments a common phenomenon is a temporary diminution or cessation of urine flow, even although the blood-pressure is raised to a considerable extent and the kidney volume markedly increased; conditions which—on the mechanical or filtration theory of urine secretion—should inevitably produce diuresis. There is, in fact, very often at first an inhibition of secretion (followed in the majority of cases by the characteristic secretory activity), even although the vascular conditions are throughout favourable to the occurrence of free secretion.* The extract is therefore liable to cause two effects which are antagonistic to one another. The most reasonable explanation of this is afforded by the supposition that the gland contains not only a substance which stimulates the kidney cells to activity but also another substance which depresses their activity, and this to so great an extent in certain cases that the kidney ceases to secrete, although all the vascular conditions for urine secretion are of the most favourable character. Nevertheless, the secretory substance usually ultimately proves the more potent: or it may be that the kidney cells are more susceptible to its influence.

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* These facts are illustrated by several of the tracings given in the paper in the 'Phil. Trans.' for 1906 by Herring and myself. This paper deals exclusively with the effects upon the kidney and urine-flow of extracts of pituitary.

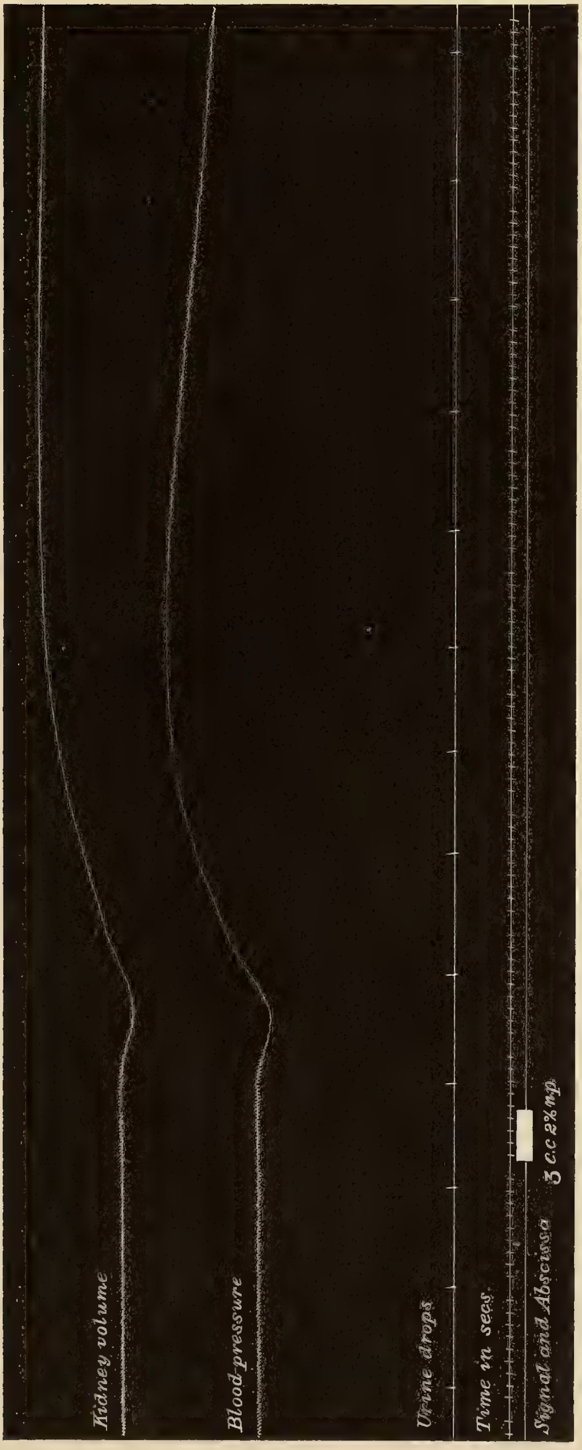

Fig. 1.—Cat: chloroform-alcohol. Tracing of kidney volume, blood-pressure and rate of flow of urine. At the place marked by the signal a small dose of extract of posterior lobe was administered intravenously.

These two substances are not the same as those which influence the blood-vessels of the kidney, which may also be affected in opposite ways. For although the most striking effect upon kidney volume is to produce augmentation, this augmentation is in a large number of cases preceded by a temporary diminution. But the temporary diminution of volume of the kidney is not the cause of the temporary diminution or cessation of urine-flow, which, as above noted, is frequently seen as the first effect of an intravenous injection; for the diminution (or cessation) of flow may last long after the diminution of volume has disappeared, and even after this has become replaced by a large augmentation of volume.*

Finally, an important fact in the physiology of the pituitary body is that which was first satisfactorily determined from the experiments of Paulesco (1907), viz., that this organ, small though it is (it weighs about ½ gramme in man), is essential to life. Animals from which it is removed were found by Paulesco inevitably to die, usually within 48 hours—indeed, sometimes within 24 hours, although in others the fatal result was delayed to three or four days. Previous observers had obtained contradictory results, some denying that the removal or destruction of the gland produces any appreciable result (Friedmann and Maas, 1900, Lo Monacho and Van Rynberg, 1901), others averring that it is fatal but that life may be prolonged after removal for several days or weeks (Marinesco, 1892, Vassale and Sacchi, 1892, Narbutt, 1903). But the methods which were adopted before Paulesco's work was published were not calculated to inspire confidence that the removal of the gland was complete, since in them attempts were made to arrive at the situation of the pituitary either through the base of the skull or through the vertex. By both methods the difficulties of the operation are very great, as are the liabilities to hæmorrhage and to injury of adjacent parts of the brain. Moreover, no clear view can be obtained of the gland by those methods, and the operator works largely in the dark. Paulesco arrives at the gland from the side through the temporal bone, which is removed freely on both sides, an incision also being made in the dura mater. Through this on the one side a retractor is introduced, and the side of the brain gently elevated until the reddish-yellow pituitary body is seen lying in the sella turcica. It can, in the dog, easily be shelled out of this with a small curette, and the brain may then be allowed to resume its normal position and the wound closed. The animals show, on recovery from the anæsthetic, at first, as a rule, no adverse symptoms and take their food readily; but in the course of the second day they begin to exhibit lassitude, and they die, without any very clear cause, within 48 hours. Paulesco performed numerous experiments on various animals belonging to different classes of vertebrata, always with a similar result. Control experiments in which there was a rehearsal of the whole operation, but without actual removal of the pituitary, produced no effect; death, therefore, must have resulted from the removal of this body.

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* These observations of Herring and myself upon the effects of pituitary extracts upon the renal blood-vessels and on the secretory functions of the kidney have been confirmed by J. Pal (1909), who states, however, that the dilatation effect is produced on the peripheral branches only of the renal arteries, the main trunks participating in the constriction which is produced in the vessels generally. Pal also finds that the coronary vessels participate in the general constriction of blood-vessels caused by pituitary extract, which in this respect also differs from suprarenal extract.

Similar experiments have been carried out in the Hunterian Laboratory of the Johns Hopkins University, by Harvey Cushing, in conjunction with Reford (1909). Their experiments have been entirely on dogs, altogether 16 in number.* The method employed is in the main that used by Paulesco, but with one or two improvements of technique. The results of complete extirpation were uniform and entirely confirm Paulesco's conclusion. Livon (1909) has also performed confirmatory experiments.†

These experiments exhibit the serious danger that lurks in any proposal for entire removal of the pituitary for tumours. It is clearly necessary that some portion be left in order that the functions of the gland, which are essential to life, may be carried on. For it has not been found possible either by grafting pituitary or by feeding with pituitary substance to sustain life after removal of this organ—as in the otherwise analogous case of complete thyroidectomy.

It is not yet known which part of the pituitary is essential to life. It is almost impossible to remove one part alone, so closely are they dovetailed into one another; indeed, the pars nervosa and pars intermedia are in direct continuity, and both are almost completely enclosed within the pars anterior.

Paulesco states that the mere separation of the pars nervosa from the infundibulum has sometimes proved equally fatal with the actual removal of the gland. In view of the discovery by Herring that the secretion of the pars intermedia discharges through the pars nervosa into the infundibulum, this statement of Paulesco is of great interest. But it still requires confirmation.

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* Cushing has since reported 100 cases of total or partial hypophysectomy, 'Journ Amer. Med. Assoc.,' July 24, 1909.—[*Note added August* 3, 1909.]

† It has recently been denied by Fichera (1906) and by Gemelli (1908) that destruction of the hypophysis is followed by a fatal result. I am convinced, however, from my own experience, that in such cases the destruction could not have been complete. My experiments in this direction, although less numerous, confirm those of Paulesco and of Cushing and Reford, but I have not thought it necessary in the present communication to refer to them in detail.

Masay (1908) has endeavoured to produce "pituitary insufficience," *i.e.* a diminution or interference with the functions of the gland, by preparing an antiserum or cytotoxine by intraperitoneal injection of a guinea-pig with an emulsion of dog's pituitary at intervals of two days, and after five injections collecting the blood of the guinea-pig, centrifugalising it, and injecting the serum (about 10 c.c.) under the skin of a dog. After two or three such injections the dogs, according to Masay, show symptoms which he interprets as due to pituitary insufficience, viz., loss of flesh, muscular weakness, especially in hind limbs, and modifications in the skeleton, accompanied by histological changes in the pituitary; the symptoms constituting, according to Masay, a veritable *cachexia hypophysipriva*. But such experiments require to be multiplied and carefully controlled before the results can be accepted as produced by changes in the pituitary body.

Various views other than those here set forth have been taken regarding the functions of the pituitary. Cyon, who was one of the first to study the effects of intravenous injections of pituitary extracts, considers that the gland secretes several active substances, one of which in particular acts upon the regulator nerves of the heart—especially the vagus—increasing the force of the beats and slowing the action of that organ, this being accompanied by a raising of blood-pressure; an action somewhat like that of muscarine. Cyon also states that direct excitation of the gland *in situ*, whether electrical or mechanical, is capable of producing effects of a similar character to intravenous injection. According to Pirrone (1903) and to Livon (1908) these effects are not due to excitation of the hypophysis but of adjacent parts of the brain or its membranes. But Cyon states that after extirpation of the pituitary the effects are not got; nor according to him is a rise of blood-pressure obtained in the carotids on compressing the aorta in a hypophysectomised animal. Masay (1908) has repeated these experiments and obtained results similar to those of Cyon, but was inclined to attribute them to operative shock, although recognising that this explanation offers difficulties. Cyon believes that the pituitary is an organ which is closely inter-related to the thyroid, being set in activity by differences of pressure within the skull, and influencing the flow of blood to the brain through the thyroid.

Rogowitz (1889) and others have regarded the pituitary as supplementary or vicarious in its functions to the thyroid apparatus, this term including the parathyroids, but it is difficult to reconcile this view with the results yielded by experiments on the effects of extracts of the two glands and on the results of extirpation.

An antitoxic function has also been ascribed to the organ, certain observers looking upon the gland as destined to neutralise poisons of bacterial origin or even poisonous substances produced by the tissues. This view was suggested, but apparently afterwards relinquished by Marie, and has been upheld by Guerrini (1904), Gemelli (1906), and Thaon (1907), who describe structural appearances in the pituitary after poisoning with bacterial products and with certain drugs which they regard as evidences of a functional reaction or hyperactivity. Such a conclusion does not, however, appear to be justified by the facts observed.

It seems, at any rate, clear that we must look upon the anterior lobe as different in function from the posterior lobe (including the pars intermedia), and it is advisable to study these parts as far as possible separately.

PRESENT OBSERVATIONS.

During the last two years I have been engaged in attempting to elucidate the question of the function of the several parts of the organ. Dr. W. Cramer at first, and more lately Dr. H. Pringle, have materially assisted me in chemical examinations connected with the research, and Dr. Pringle has given me help in various other ways, including the histological examination of the pituitaries. I am indebted to Messrs. Burroughs, Wellcome, and Co. for a supply of material in the shape of pituitaries in both the fresh and prepared condition.

Most of the pituitary material used has been obtained from fresh ox pituitaries which had been kept for a few days (while being collected and sent) in a bottle with chloroform. Each gland was then taken, its connective tissue capsule removed, and the larger anterior part separated from the much smaller posterior part—the latter including also the pars intermedia. The separated portions were spread thinly on glass and dried thoroughly on a warm plate at a temperature of about 40° C., and in this state were powdered and preserved.

ANTERIOR LOBE.

Feeding Experiments.

With this material a series of feeding experiments has been planned and in part carried out on white rats, both young and adult, the animals being usually kept three or four together in a cage, with a similar number, generally from the same litter, in a second cage as a control. The cages are contrived so that the urine and fæces are separately collected, and are contaminated as little as possible with the food, which has consisted of bread and milk in a certain constant proportion made into the consistency of a thick paste. This paste is placed within a cylindrical beaker. Upon the top of the paste, but within the beaker, rests a heavy metal disk with a central hole large enough for the snout of a rat to pass through. By this means the animals are permitted to feed at any time, the metal ring always falling as the food decreases, so that the rats cannot scatter the food over the cage as otherwise they are apt to do. The amount of food consumed was determined (in some of the experiments) each day by weighing the beaker and its contents. The urine was allowed to accumulate in a vessel containing abundance of thymol for a regular number of days—usually four or seven—and was then examined for amount, reaction, specific gravity, percentage of urea, and in some cases for phosphorus content. The fæces were removed daily and reserved when it appeared necessary; they showed no difference obvious to the eye between those of the pituitary-fed and control animals. The pituitary-fed rats received with their bread and milk a certain small but constant amount of the dried gland, either from the anterior or from the posterior lobe, the proportion of pituitary substance to the bread and milk food being extremely small. The control animals were fed and kept in a completely similar fashion, except that in place of pituitary an equal amount of the dry powdered substance of some other gland—usually testicle or ovary—was added to the bread and milk. The weight of the animals was regularly recorded.

Some of the details of one experiment performed in this manner may here be given. A litter of eight rats was taken immediately after being weaned and divided into two groups (A and B) of four each; the two groups being, as it happened, of exactly the same weight. All were males with the exception of one of the rats of Group B. Both groups were at first put upon the bread and milk diet alone for four days; it was found that during this period Group B tended to increase in weight slightly faster than Group A. To the diet of Group A was then added a small constant amount of the dry powdered material derived from the anterior lobe of ox pituitaries. Group B, which were kept as controls, received in place of this an approximately equal amount of dried powdered material prepared from the testicle; for which was, later, substituted a similar material prepared from ovaries.* At an advanced period of the experiment a small amount of powdered material derived from the posterior lobe of the pituitary was mixed with the powdered anterior lobe which was being given to Group A. The experiment was started on March 4 of this year and terminated on June 3.

At the commencement of the experiment the average weight of each rat was 44·25 grammes in each group. A week before the termination of the experiment (*i.e.* on May 27) one rat of Group A and the female of Group B were killed. At this time the average weight of each rat of Group A was 160 grammes; of each rat of Group B, 131 grammes. After eliminating the female, which weighed only 113·5 grammes, and one of the (male) rats of Group A, which weighed 170 grammes, the three remaining (male) animals belonging to Group A were found to average 165 grammes; while the three remaining (male) animals belonging to Group B averaged 142 grammes.

At the termination of the experiment the remaining animals, which were all healthy, vigorous males, were killed and X-ray photographs were taken of them. These have yet to be examined and measured.

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* The amount of calcium in the substances given to the two groups was nearly the same: if anything the balance was against that given to Group A.

Table showing Results of Pituitary Feeding in White Rats. Two groups (A and B), each containing four young rats, all of the same litter, were placed under similar conditions of diet, except that to the food of Group A a small constant proportion of dry powdered anterior lobe of ox pituitary was added, whilst Group B received a similar amount of testicular or ovarian substance. The addition of these substances to the food was begun on March 8.

Date.	A.					B.				
	Weight of animals.	Amount of urine collected.	Percentage of urea.	Sp. gr.	Reaction.	Weight of animals.	Amount of urine collected.	Percentage of urea.	Sp. gr.	Reaction.
	grammes.	c.c.				grammes.	c.c.			
March 4	133	—	—	—	—	133	—	—	—	—
8	141	76	2·7	1030	acid	156	80	2·4	1030	acid
12	184	100	2·65	1033	acid	198	115	2·5	1030	acid
16	227	82	3·2	1040	alk.	227	82	3·2	1040	alk.
20	269	125	2·8	1033	neutral	255	128	2·75	1035	neutral
24	284	134	2·71	1033	alk.	269	127	2·83	1034	alk.
28	325	95	3·66	1043	alk.	312	103	3·83	1040	alk.
April 1	346	220	2·16	1026	alk.	318	196	2·37	1027	alk.
4	369	—	—	—	—	325	—	—	—	—
8	383	330	2·66	1027	alk.	354	340	2·16	1025	alk.
15	439	272	3·5	1033	alk.	411	280	3·3	1033	alk.
22	454	280	3·3	1036	alk.	397	225	2·66	1036	alk.
29	496	208	—	—	—	425	210	—	—	—
May 6	539	210	2·16	1033	alk.	439	190	2·08	1037	alk.
13	510	210	1·91	1030	alk.	425	160	2·00	1035	alk.
20	567	338	2·5	1032	alk.	482	293	2·16	1034	alk.
27	638	353	2·83	1033	alk.	524	225	2·95	1035	alk.

Up to April 1 the urine was collected every four days, after this every seven days. On May 27 one rat of each group was killed. The experiment was continued to June 3 with three rats only in each group. This part is omitted from the table.

The experiment is here set out in tabular form and some of the results are given in the form of charts (figs. 2 and 3). In the table the total weight of the animals in each group, the total amount of urine collected in the given time, its reaction and specific gravity, and the percentage of urea are shown.

The chart (fig. 2) shows the relative rate of increment of weight of Groups A and B, the dates being marked upon the abscissa at proportionate intervals, whilst the weights in grammes are given as ordinates. The drop in weight from May 6 to May 13 was due to the fact that the feeding of the animals during part of that time was restricted to only two hours a day. This chart shows that whereas before the pituitary feeding began there was a tendency for the rats belonging to Group A to increase in weight less rapidly than those of Group B, after eight days of the addition of pituitary to the food of A these show a steady increase on Group B, the increase being continued up to the end of the experiment.

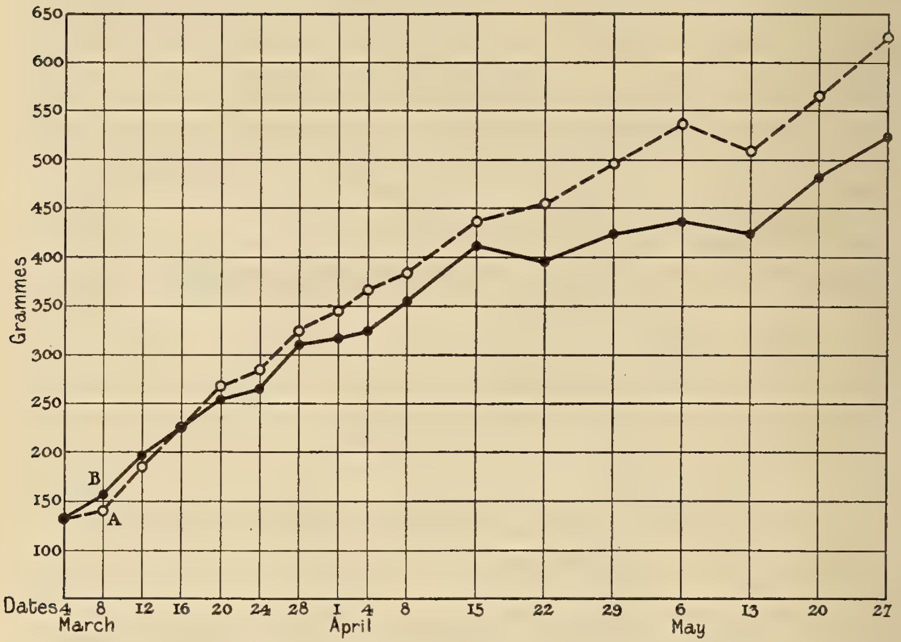

Fig. 2.—Chart showing Rate of Growth of two groups of Rats from same litter. Group A with addition of anterior lobe of pituitary to food ; Group B, controls. The addition was commenced on March 8.

The chart (fig. 3) shows the relative amount of urine per diem in the two groups, calculated per kilogramme weight of animal. The ordinates represent cubic centimetres: the dates as before are marked proportionately on the abscissa. The correspondence between the two curves is almost complete: the fluctuations, which are considerable, and are probably caused by varying meteorological conditions—especially, perhaps, temperature—synchronise in a singular manner:* only towards the end of the experiment and after the animals of Group A had received a certain small proportion of posterior lobe, intermingled with the dried anterior lobe powder which they had previously been taking, is there any distinct difference in the relative amount of urine per gramme weight of animal, and even this does not show itself immediately.

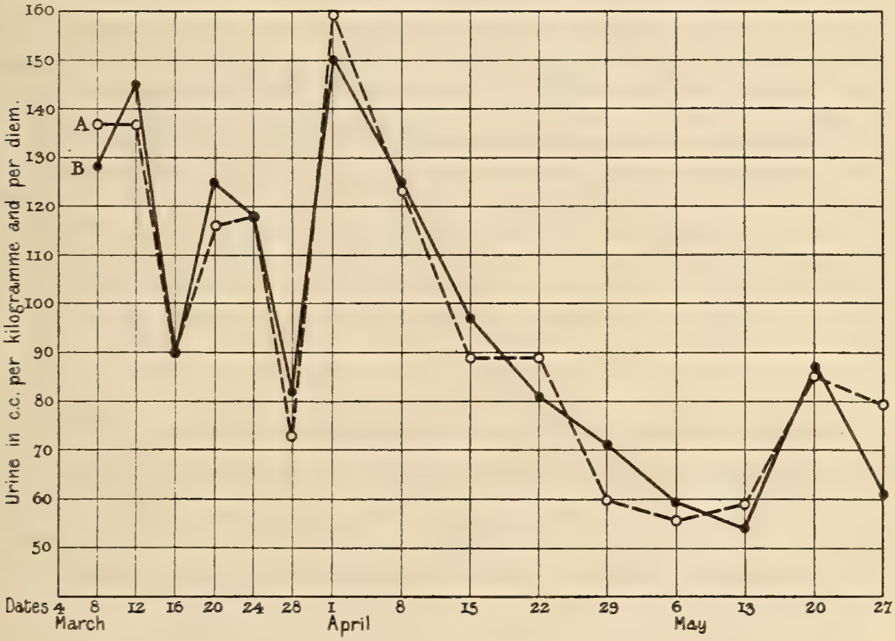

Fig. 3.—Chart of Urine Secretion of the Groups A and B.

The fluctuations in the urea excretion correspond on the whole with those of the amount of urine, but there was less urea excreted in the pituitary-fed animals than in the controls.

This experiment is given to show the manner in which we are endeavouring to approach the problem as to the effects of pituitary feeding upon growth rather than with the intention of drawing positive conclusions from it. We must await the result of other experiments still in progress

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[*] The necessity of working with a duplicate set of control animals is well illustrated by the curves in this experiment. Without such control the very considerable fluctuations, which are probably due to meteorological conditions, might easily be misinterpreted as effects contingent on the experiment.

and projected before attempting to decide whether pituitary feeding has the distinct influence upon growth which might be inferred from this experiment.

Here the observations of Cerletti (1907) and of Sandri (1907) may be briefly referred to.

Cerletti injected young animals (intraperitoneally). As the result he found that the bones of the animals receiving the pituitary emulsion were after some time, as compared with controls, somewhat shorter as regards the diaphyses but larger as regards the epiphyses. If Cerletti's results are to be accepted, they appear to indicate if anything a retardation in growth of the bones, at any rate in the direction of length. Sandri fed young mice with pituitary—apparently with the pure crude gland to the exclusion of other food. He states that this caused an arrest of growth, but there seem to have been no controls made with food of a similar percentage composition. Sandri also injected young guinea-pigs with an emulsion of the gland, and found that in these also growth was diminished.

Our experiments have certainly not shown any arrest of growth as the result of pituitary feeding.

Grafting Experiments.

Another method by which we are endeavouring to investigate the effects of pituitary secretion upon growth is that of implanting pituitaries of other individuals of the same species in various parts of the body, such as the brain, the subcutaneous tissue, the muscular tissue, the peritoneal cavity, and the kidneys. But so far these experiments have failed to throw any clear light on the question by reason of the fact that we have not in any case obtained a permanent graft of the implanted organ. The chief result which has been noted is a temporary increase in the amount of urine secreted, a result due either to absorption of the diuretic substance which the transplanted pituitary contained, or, perhaps, to a temporary functioning of the implanted organ preceding its degeneration. All the animals in which this attempt to implant the pituitary has been made—including dogs, cats, monkeys, and rats—have remained healthy and have been killed after a certain lapse of time. In no case have we been able on *post-mortem* examination to substantiate the presence of the characteristic epithelial structure of the pituitary at the site of implantation. In one experiment, which is still in progress and a chart of which is appended (fig. 4), the animals (rats) with pituitary grafts grew at first at exactly the same rate as the controls of the same litter and sex. But after three weeks the controls for some unexplained reason lost weight for a few days, and have hardly as yet managed to catch the others up.

The chart of urine-secretion (fig. 5) follows almost exactly the same course in the two groups: the effect of the implantation in these animals was therefore to all appearance *nil.*

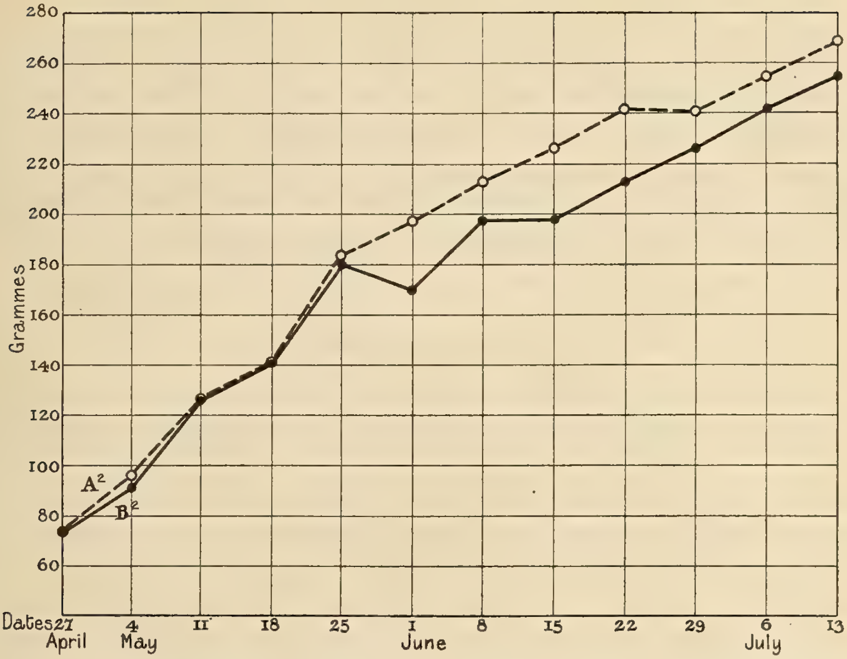

FIG. 4.—Chart showing Rate of Growth of two groups of Rats, A^2 and B^2, of the same litter, A^2 with attempted pituitary implantations; B^2 controls. The implantations were made on May 4.

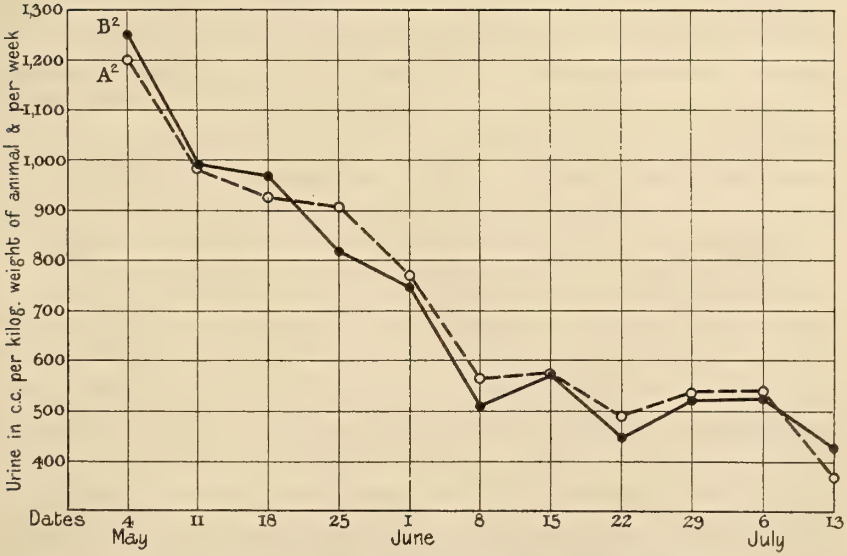

FIG. 5.—Chart of Urine Secretion of the Groups A^2 and B^2.

POSTERIOR LOBE.

The experiments hitherto mentioned have been concerned mainly with an attempt to elucidate the functions of the anterior lobe of the pituitary; those that we have next to consider had mainly in view the diuretic properties of the secretion of the gland, which former investigations have shown to be most probably connected with the posterior lobe, at least with the pars intermedia. The experiments on this subject may be referred to under three heads, viz., feeding, grafting, and stimulation by injury.

Feeding Experiments.

There seems to be little doubt that the exhibition by the mouth of an active water-extract of the posterior lobe may greatly increase the amount of urine secreted. We have frequently, although not invariably, obtained such increase in animals; where it has failed to occur—as, indeed, may happen also with intravenous injection—the failure is probably due to some special condition in the individual which renders him irresponsive to the excess of pituitary substance which is passing into the blood. What this condition may be we need not stop to inquire, but it is noteworthy that the activity of the gland in promoting diuresis often appears greatest in cases in which the amount of urine which was previously being passed is less than usual.

In connection with this part of the investigation, Mr. Harold Stiles was good enough to allow an active extract of the posterior lobe of the ox pituitary to be tested upon two children under his care, both convalescent after operations and otherwise in good health; these may be given as instances of the effect of the extract.

In the first of these cases, a boy, aged 10 years, during a period prior to the exhibition of the extract, was secreting an average of 28 fl. oz. of urine per diem, while during and immediately after the period that the extract was being administered the average secretion was 38 fl. oz.

In the second case, that of a girl, aged 9½ years, the average amount of urine in the period prior to the administration was unusually low, viz., only 9 fl. oz. per diem, whereas during and immediately after the period of administration it rose to an average of 29 fl. oz., and was one day as much as 35 fl. oz.

Instances of marked increase of urine-secretion as the result of the clinical administration of pituitary extract exist in the literature of the gland. Marinesco (1895) gives the results of the treatment of three cases of acromegaly with tablets of pituitary. In the first the average amount of urine was increased from about 1 litre to 1½ to 2 litres per diem; in the second from 1100 to 1300 c.c.; and in the third case, which was already diabetic, the increase was from 16 to 21 litres. J. Azam (1908) observed a marked diuresis as one of the effects of administration of 0·3 to 0·4 gramme ox pituitary in cases of infectious fevers.

The accompanying chart (fig. 6) of a feeding experiment upon rats may also be here given in illustration of the diuretic effect of pituitary feeding.

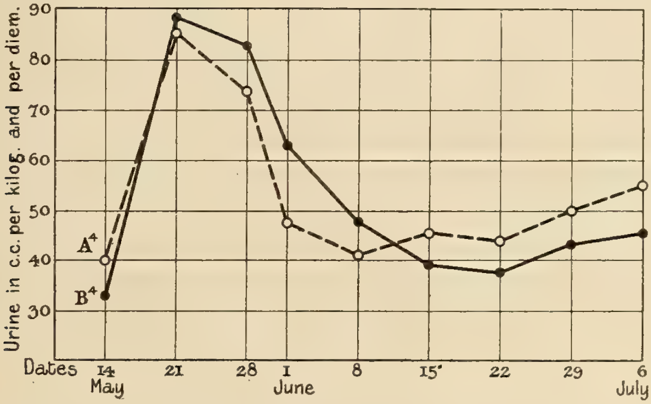

Fig. 6.—Chart of Urine Secretion of two full-grown Male Rats, A^4 and B^4, fed on bread and milk, to which in the case of A^4 a small addition of dry sheep's pituitary was made on and after June 1, while B^4 was given as a control a similar addition of dry testicular substance.

The chart shows the amount of urine in cubic centimetres per kilogramme and per diem secreted prior to and during the administration (along with the ordinary bread and milk food) of a small amount of sheep's pituitary. In this case the whole gland was used, a little of the dry powder, which had been kept for several years, being added to the food. Two large male rats were chosen of about the same weight (250 grammes), one for use as a control. To the food of this one an equal amount of dry testicular substance was added during the time that the other one was receiving pituitary substance. A preliminary observation was first made, extending over rather more than two weeks, no addition being made to the ordinary food. As a result of this it was found that one of the two rats secreted rather less urine per kilogramme body-weight than the other. This one (A^4) was selected for the pituitary addition, and the other (B^4) for the control. During the first week of pituitary feeding the curve of the urine secretion of A^4 is approaching that of B^4; during the second week it crosses it, and in the subsequent weeks it maintains a higher position, so that in place of secreting about 25 per cent. less urine than B^4, as was the case before the feeding with pituitary began, it now secretes about 25 per cent. more. The same fact is illustrated towards the termination of the experiment which is illustrated by the chart given in fig. 2.

Grafting Experiments.

The effects upon the secretion of urine of grafting pituitary have already been referred to in connection with growth, but it may be of interest to record some of the results on urine-secretion which have been yielded in our attempts to effect the implantation.

A cat, which was passing, prior to the operation, 207 c.c. of urine per diem (average of 15 days), was found to pass during the 15 days succeeding the implantation of the pituitary of another cat into its peritoneal cavity an average of 276 c.c., the greatest increase being during the first week after the operation. In another cat the average amounts were 180 c.c. (before) and 233 c.c. (after). In a monkey the amounts recorded were 202 c.c. and 255 c.c. respectively—for a daily average during 16 days before and after the implantation. Of two rabbits operated upon in this way, the effect produced in one was hardly noticeable, but in the other the amount of urine rose

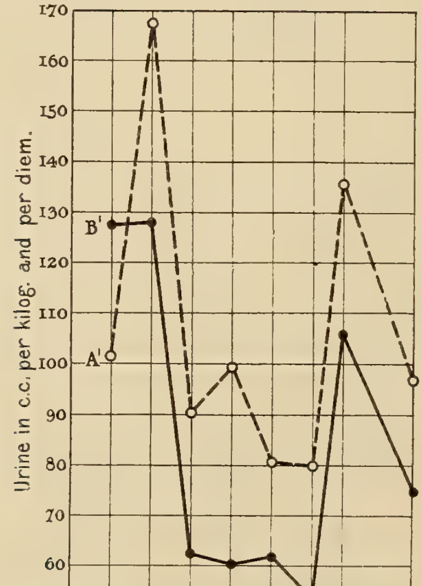

Fig. 7.—Chart of Urine Secretion of two groups of full-grown Rats (three in each group): Group A^1 with pituitary implantation into muscles of back; Group B, controls. The implantations were made on March 9.

from an average of 50 c.c. before the implantation to an average of over 100 c.c., and on one day to 150 c.c. during the week following.

The result was in all these cases temporary, and the effect of implantation upon the urine gradually disappeared, no doubt coincidently with the destruction and absorption of the implanted tissue, which, as I have already mentioned, we failed to find on *post-mortem* examination.

Similar results have been obtained with rats. Thus in one experiment a graft of pituitary was made into the muscles of the back in three full-grown rats, three others of similar dimensions being employed as a control. The amount of urine passed before the operation was rather more in the control animals than in those selected for the implantation. After the grafting the amount of urine per gramme weight of animal showed a decided increase, and this increase persisted for some weeks. This is shown in the accompanying chart (fig. 7), in which the curve A^1 shows the amount of urine in cubic centimetres per kilogramme weight of the pituitary-grafted animals, and curve B^1 the amount in the controls. The urine was collected at first every four days, subsequently with a seven days' interval.

Stimulation of Pituitary by Injury.

With the view of determining what effect injury to the pituitary might have upon the performance of its functions, we have in some animals exposed the gland by the method of Paulesco and subjected it either to mechanical injury or to partial destruction by means of a feeble thermo-cautery. Before the operation the animals were all approximately in nutritive equilibrium. The results are striking, and may be illustrated by giving the main results of three experiments, all on dogs.

(*a*) A dog weighing 5·5 kilogrammes and taking each day 180 grammes of dog biscuit was passing, immediately before the operation, from 30 to 40 c.c. of urine per diem. The pituitary was subjected to partial injury by means of a warm, but by no means hot, electro-cautery; the animal recovered without a bad symptom, except that during one or two days it was thought to turn towards the operated side in walking. The day after the operation 40 c.c. of urine was collected; the next day 180 c.c.; the next 230 c.c.; the next 102 c.c., the amount gradually coming down, although an average amount of 114 c.c. was maintained during the whole period of 19 days that the animal was kept alive, whereas the average of the 19 days prior to the operation was 54 c.c. It may be noted that, contrary to what occurred in the other cases, the amount of water consumed was in this case greater during the after period than during the period prior to the operation.

(*b*) A dog weighing 9·5 kilogrammes was passing, during 11 days prior to the operation (which was similar to the last), 110 c.c. urine per diem. During the 11 succeeding days the average was 182 c.c., and the increase was maintained until the animal was killed 10 days subsequently. The greatest amounts passed were on the third, fourth, and fifth days after the operation, the amounts on these days averaging 266 c.c. The average daily amount of water taken for the 11 days before operation was 400 c.c., for the 11 days after operation 310 c.c.

(*c*) A dog weighing about 6 kilogrammes had its pituitary exposed by the method of Paulesco and Cushing, and with a blunt instrument the gland was mechanically injured and partially broken. During the four days preceding the operation the average daily amount of urine secreted was 119 c.c. During the four days subsequent to the operation the average amount was 192 c.c. After the fourth day in this case the average fell to a normal amount. The average daily amount of water taken during the four-day periods was 322 c.c. before and 235 c.c. after the operation.

(*d*) Another dog weighing about 9 kilogrammes was subjected to exactly the same operative procedure, the brain being exposed from the side and raised so as to bring the pituitary body freely into view. But the gland was not touched nor intentionally injured in this case. The wound was closed in the same manner as in the dogs in which the pituitary had been mechanically injured. The polyuria which was displayed in the other three cases was only exhibited on the second and to a less extent on the third day in this dog, and this is probably to be accounted for by the fact that the animal refused its ordinary diet of dog biscuit and water on the day following the operation and was given milk instead: of this it consumed 450 c.c., whereas the amount of water which it was in the habit of taking with the biscuit rarely exceeded 200 c.c.

The microscopical examination of the pituitary in the first two dogs (*a* and *b*) reveals no serious injury, but in both blood is extravasated into the central cavity, and there is marked increase of the colloid secretion of the pars intermedia—which previous experiments have shown to be probably associated with the diuretic function of this gland. The pituitary of the third dog has yet to be examined, but there is no doubt about its having been injured.

The bearing of these results upon the polyuria which occurs in injuries and tumours affecting the base of the brain is of considerable clinical interest. Such cases are well known to surgeons and physicians, and are frequently recorded. The polyuria and glycosuria have generally been set down to injury of a hypothetical centre at the base of the brain. Even when associated, as these symptoms often are in acromegaly, with tumours of the pituitary, they have not been usually ascribed to an increased activity of that gland.* Indeed, in many cases of acromegaly, polyuria does not occur. Doubtless this is due to the hypertrophy and increased activity being confined to the anterior lobe, which is the part usually involved in this disease. Often it does not occur until the disease is more advanced, and may then be due either to the hypertrophy involving the pars intermedia or to this part being stimulated mechanically by the adjacent growth.

Conclusions.

1. The pituitary body consists of three parts: (1) the pars anterior, formed of vascular glandular epithelium; (2) the pars intermedia, formed of a less vascular epithelium secreting "colloid"; (3) the pars nervosa, consisting mainly of neuroglia, but invaded by the colloid of the pars intermedia, which passes through it into the infundibulum of the third ventricle. These parts differ from one another in function.

2. The function of the pars anterior is probably related to growth of the skeletal tissues, including cartilage, bone, and connective tissue in general. The chief evidence in favour of this is derived from the fact that hypertrophy of the pars anterior is associated with overgrowth of the skeleton and of the connective tissue in growing individuals, and of the connective tissues especially in individuals in whom growth has ceased. These effects are probably produced by hormones.

3. The function of the pars intermedia is to produce a "colloid" material which contains active principles or hormones acting upon the heart, blood-vessels, and kidneys. Probably there are several such hormones acting upon blood-vessels and kidneys independently, and also acting antagonistically; so that according to circumstances either a rise or fall of blood-pressure, an increased or diminished secretion of urine, may be produced, and the effects on the kidney may be independent of those on the blood-vessels. The hormones which appear to be most active are those which produce contraction of the blood-vessels in general, with dilatation of the renal vessels and

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* Rosenhaupt (1903), who describes a tumour of the pituitary associated with polyuria, states that he is "loth to assume that this is due to the pituitary tumour, since there are no physiological grounds to support such a view." And Steinberg (1897) remarks that in the "die alte Casuistik" it was not uncommon to associate pituitary tumours with polyuria and glycosuria, but that it is more probable that the latter symptoms are due to an overlooked condition of acromegaly. More recently, Borchardt (1908) has suggested that the glycosuria which is so often recorded in acromegaly may be associated with the hypertrophy of the pituitary, but does not especially connect it with the pars intermedia.

increased activity of renal cells, but there appear to be others which cause constriction of renal vessels and diminished activity of renal cells; the effects of these latter are generally less lasting. There is also usually an inhibitory effect produced on the heart.

4. Extirpation of the pituitary body is incompatible with survival during more than two or three days. Injury of the organ when not extensive causes no pronounced symptoms other than increased secretion of urine, which is accompanied by increased production of colloid by the pars intermedia. Complete removal of a pituitary tumour in man should not be attempted, since entire removal of the gland would in all probability be speedily fatal.

5. Acromegaly and gigantism appear to be due to an increase of function of the anterior lobe alone. It is this lobe which is always in the first instance hypertrophied in those affections. If the posterior lobe is involved polyuria is likely to result. The fatal termination which ultimately occurs in acromegaly—but which may be long deferred—is probably associated with a change in the nature of the tumour, which from being a mere glandular hyperplasia becomes of a sarcomatous nature, while the normal tissue becomes destroyed.

6. The addition of a small but regular amount of pituitary substance to the food produces an increase in the amount of urine secreted. This effect is obtained from the pars intermedia and posterior lobe, not from the anterior. Implantation of the pituitary of another individual of the same species may produce a similar effect on the urine, causing an increase of secretion which may last a short time but soon disappears.

7. The addition of a small amount of pituitary substance to the food appears to favour the growth of young animals: it does not impede or restrict their growth. The attempts at implantation of pituitary in young animals have not in these experiments been followed by any deterioration in growth as compared with controls; if anything, there are signs of improved nutrition. But we have not succeeded in establishing permanent grafts, and any result which might be looked for could only be of a temporary character.

LITERATURE.

A very full bibliography of the subject of the pituitary body and of acromegaly in relation to it is given by Masay in his thesis (see below), published in 1908. The following papers are more particularly referred to in the preceding pages—some of these are subsequent to Masay's thesis :—

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On the Occurrence of Protandric Hermaphroditism in the Mollusc Crepidula fornicata.

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Introduction.

Crepidula fornicata is a streptoneurous Gastropod belonging to the Calyptræidæ, a family of the Tænioglossa. It was first introduced into England from America about 1880 (1), when it was imported with American oysters. In America it is found on the east coast from Labrador to Florida, but in England so far as is known, it is confined to the Essex and Lincolnshire coasts, occurring, however, in abundance in shallow water in the neighbourhood of the mouths of the Crouch and Blackwater rivers. The conditions on the Essex coast seem to be highly favourable for its growth and propagation; indeed, so favourable, that within five or six years it has over-run the oyster beds at West Mersea. By attaching themselves very strongly to oyster-shells they cause the oyster fishermen much trouble, and it may be remarked, by competing for food and oxygen with the oysters may become a cause of much more serious trouble in the future. To obtain food the animals raise the anterior part of their shell, and extending the head to the front edge of the shell, move it slowly from side to side: at times the whole shell may be similarly turned slowly round to the one side or the other.

Crepidula fornicata is sedentary for the greater part of its life. It forms "chains," as Prof. Conklin calls them, by the curious habit the individuals have of fixing themselves in linear series one on the top of another as in fig. 1. Chains of as many as 12 individuals have been found. Viewed as a

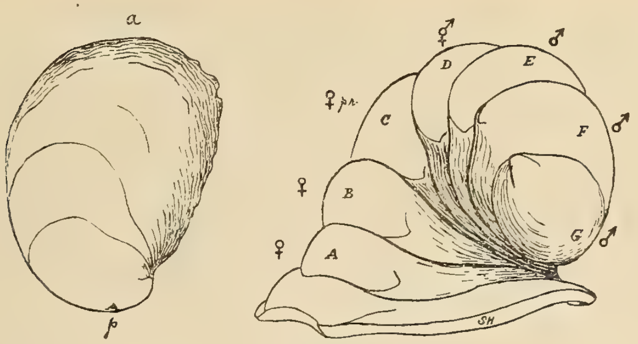

Fig. 1.—On the left; Dorsal View of a single Shell: *a*, anterior; *p*, posterior. On the right; a Postero-dextral View of a Chain attached to an Oyster-shell (SH). ($\frac{3}{4}$ nat. size.)

A, shell of proximal individual; B, shell of second individual, and so on. The symbols adjacent to the shells denote the secondary sexual characters of the inhabitants (see text).

whole, a chain is seen to form a spiral of about half a turn, bending over to the right.

On close examination of the chains it is found that when an individual settles down upon another it places the right antero-lateral border of its shell close to or touching that of the individual upon which it settles; since, however, the individual shells are roughly semi-ellipsoidal in shape, with the longitudinal axis a little concave to the right, and have the left side a little more convex dorso-ventrally than the right, it follows that the chain must form a right-handed spiral.

In a typical chain the twisting is accentuated by the gradual decrease observable in the size of the shells: the shell of the bottom or "proximal" individual being the largest, and that of the top or "distal" individual the smallest. The "proximal" individual always fixes the chain to some surface, as the shell of a dead or living animal, a pebble, or a piece of broken pot or glass. In America, chains of two or three individuals are found on the ventral surface of the King-crab (2, p. 10).

The following facts indicate that when a chain is once formed, none of the individuals separate:—

(1) The accurate fitting of each shell into the crevices and irregularities of the surface or shell upon which it occurs; hence, only short periods of separation of the individuals could be possible; no such periods have, however, been observed.

(2) In soft rock the proximal individuals wear a deep impression of the edge of the shell by the lateral movement executed in the search for food. In these cases the surface to which the middle of the foot is attached is not worn down, so that the animal becomes fixed on a boss of rock, which thus fits loosely into the aperture of the shell.

(3) Animals detached from a surface are apparently incapable of refixing themselves after a certain age, which I have not determined; for, immediately an animal is detached the sucker-like foot becomes "cupped" by a strong contraction of its muscle fibres; subsequent relaxation of the fibres does not seem to be possible.

(4) Prof. Conklin states that old individuals sometimes become permanently fixed by a calcareous secretion of the foot (2, p. 11).

There would seem to be no doubt, therefore, of the permanence of the chains. All the young ones, however, are motile, moving about by alternate extensions and contractions of the flat muscular foot.

Crepidula fornicata has hitherto been described as diœcious, with a "marked sexual dimorphism" (2, p. 16), the males having been estimated by Prof. Conklin as being on the average three-quarters the size of the females. Those individuals were apparently called males, which had a muscular, cylindrical, and tapering outgrowth, the penis, on the right side of the head just behind the tentacle, as in fig. 2, ♂. Individuals having no

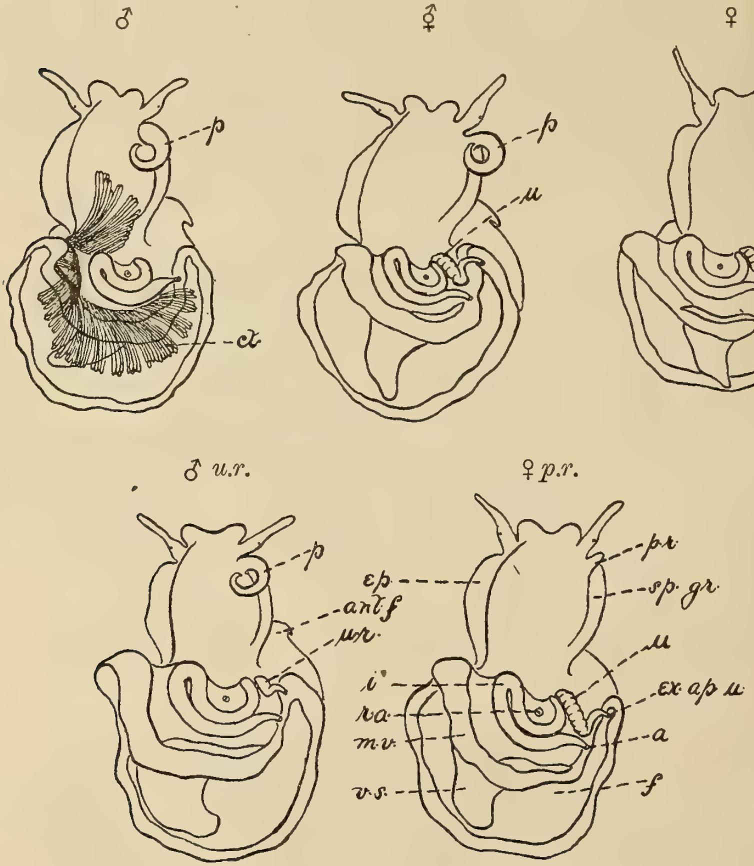

Fig. 2.—Illustrations of the Five Categories of Individuals in *C. fornicata.*

The animals were taken out of their shells and drawn from life (dorsal view), the mantle being turned back over the visceral sac. The branchial filaments are drawn only of the male. *p.*, penis; *u.*, uterus; *p. r.*, rudimentary penis; *u. r.*, rudimentary uterus; *a.*, anus: *ant. f.*, anterior part of foot; *c.t.*, branchial filaments; *ep.*, epipodium; *ex. ap. u.*, external aperture of uterus; *f.*, foot; *i.*, intestine; *m. v.*, mantle vessel; *r. a.*, external renal aperture; *sp. gr.*, sperm groove; *v. s.*, visceral sac. (Nat. size.)

such outgrowth on the head, but possessing a slightly spirally constricted tube, the uterus or external part of the oviduct, projecting on the right side of the mantle, were apparently called females (fig. 2, ♀). In dissecting a number of these animals I came across an hermaphrodite form, that is a form possessing both penis and uterus as in fig. 2 ⚥.

Before the commencement of this work, Prof. Dendy had kindly given me a large collection of the radulæ of *Crepidula fornicata* obtained by him at West Mersea, suggesting that I might investigate and compare the variability of the English and American stocks with respect to radular characters. I took the matter up, but after reading Prof. Conklin's work (2, pp. 19 to 25), on the genus Crepidula, decided to extend the investigation. Fresh material was obtained from West Mersea, and all of this was preserved in order to permit of correlation of all characters.

It was thought that the chain relations of individuals, and the sex relations in the chain would be interesting. A few *entire* chains were therefore preserved, and the relative position of the individuals recorded: thus the sex relations of the individuals in the chains were brought out as displayed in Table I.

Table I.

Ref. No.	A.	B.	C.	D.	E.	F.	G.	H.	I.
Chain 1	♀ ...	♀ ...	♀ ...	♀ ...	⚥ ...	♂ ...	♂ ...	♂ ...	♂ :
„ 2	♀ ...	♀ ...	♀ ...	♂ ...	♂ ...	♂ ...	♂ :		
„ 3	♀ ...	♀ ...	♀ ...	♀ ...	♀ ...	⚥ ...	♂ ...	♂ ...	♂ :
„ 4	♀ ...	♀ ...	♀ ...	♂ ...	⚥ ...	♂:			
„ 5	♀ ...	♀ ...	♂						
„ 6	♀ ...	♀ ...	lost ...	♂ ...	♂ ...	♂ ...	♂ :		

In Table I the individuals in a chain are denoted by the letters, A, B, C, . . . etc., the proximal individuals being denoted by A, the next to the proximal by B, and so on. The chains therefore, read horizontally:—

Forms with a well-developed penis are represented by the symbol ♂
„ „ uterus „ „ ♀
„ „ penis and uterus „ ⚥

If the chains were found to be naturally complete,* two dots are placed after the last symbol, thus—♂ :

If the chain were doubtfully complete, one dot was placed similarly, thus—♂ ·

If the chain were known to be incomplete, only the symbol was used—♂

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* Usually, in a naturally complete chain, the "distal" individual is a very young one. If, however, the distal individual is large, one can generally tell if it has had another individual on its back by the absence of the periostracum in an elliptical area on the right side of the shell.

A glance at the Table shows that all the individuals at the proximal ends of the chains are females, all the more distal individuals males, and those in the middle of the chain hermaphrodites. In discussing the records with Prof. Dendy, it occurred to us that the hermaphrodite condition appeared to be a stage in the life-history of all the individuals, and that as the males were the youngest, the hermaphroditism, if successive, must be protandric. Prof. Dendy suggested that an examination of young specimens would settle the matter. I immediately examined a collection of 48 young ones, and found them to be all males. Subsequently, 1000 young ones, namely, motile forms of the average size of about 1 cm., have been examined and found to be all males.* In a few cases the penis is a mere protuberance behind the tentacle, but as these are the smallest individuals—even larger ones requiring microscopical examination—they are doubtless the youngest males.

Sex Relations in the Chains.

A systematic examination of the sex relations of the individuals in the chains was begun at this stage. Observations of about 350 chains were recorded. A sample of the records made is shown in Table V (p. 476).

At the outset of the examination, however, it was found necessary to adopt additional categories to the ♂, ⚥, and ♀; for all stages were found on the one hand between ⚥ forms and ♂'s with a rudimentary uterus, and on the other hand between ⚥ forms and ♀'s with a rudimentary penis. The number of stages found, however, intermediate between ♂ and ⚥ was small compared with the number of stages found intermediate between ♀ and ⚥.

The following arbitrary categories were adopted:—

Males with a rudimentary uterus are represented by the symbol..	♂ *u.r.* (= uterus rudimentary). See fig. 2, ♂ *u.r.*
Forms intermediate between ♂ *u.r.* and ⚥ are represented by the symbol	⚥ *u.s.* (= uterus small).
Females with a rudimentary penis are represented by the symbol..	♀ *p.r.* (penis rudimentary). See fig. 2, ♀ *p.r.*
Forms intermediate between ⚥ and ♀ *p.r.* are represented by the symbol	⚥ *p.s.* (= penis small).
Occasionally individuals were found with both a small uterus and a small penis; such are represented thus	⚥ $^{p.s.}_{u.s.}$ (= penis small and uterus small).

In the records will be noticed here and there symbols which are bracketed, and another symbol or series of symbols placed alongside, and enclosed with these in a square bracket, thus:—[(♂)(♂)♂]. This indicates that younger and smaller individuals settled down on members of the main or primary chain, and formed secondary or side-chains; occasionally even tertiary chains were found.

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* As determined by the possession of a penis.

A comparison of the chains, even in Table V, brings out the regularity of the positions in which the different categories of individuals occur. As before, the ♀'s are found at the bottom of the chains, the ♂'s at the top, and the ⚥'s in the middle. Between the more distal ♀'s and the ⚥'s are found the ♀ *p.r.*'s; between the more proximal ♂'s and the ⚥'s or distal females are found the ♂ *u.r.*'s.

Hence, in a typical chain a series may be constructed to read from the top to the bottom, thus:—♂, ♂ *u.r.*, ⚥ *u.s.*, ⚥, ⚥ *p.s.*, ♀ *p.r.*, ♀.

It is interesting to note this series is just what one would expect to find if ♂'s passed successively through the different stages indicated, becoming finally ♀'s. It is certain, as will be shown later, that such a change does occur.

All lengths of chains occur from as many as 12 individuals in a chain down to one. A single individual was regarded as settled if its shell fitted accurately the irregularities of the surface upon which it was found, and if this surface were found to be clean. It is to be remembered that the records do not bring out the facts that in *all* chains there is typically a decrease in size from proximal to distal individuals.

In reviewing the records it is seen that a chain of a given length may be formed by various combinations of the five sex-forms (see chains 91, 338, 340, in Table V, p. 476), but the relative positions in the chains of individuals of the different categories are, with rare exceptions, constant. Occasionally it was found that an ⚥ form occurred between two ♂'s.

Chain Formation.

From a study of the records therefore, it would seem that chains are formed as follows:—

A male settles down on some surface, but before another male creeps on to its back it may pass through the series of changes from ♂ to ♀ as shown in Table II. Chains of one individual may be said to be at Stage I, chains of two individuals at Stage II, and so on.

At any time while the single male is changing into a female, another male might creep on to its back, settle down permanently, and form a Stage II chain. The new comer may then change to a female, and thus a Stage II chain might be found in any of the conditions represented in Table III. Similarly, another male might settle down upon a Stage II chain at any condition of the latter, and, changing in turn into a female, would form a

Stage III chain in one of the conditions represented in Table IV. Similarly, Stages IV and V, and so on, would be formed, and tables could be drawn up to indicate their different possible conditions.

Table II.*

	A.	B.	No of times found among records made.
Stage I (*a*) ...	♂ :		51
,, (*b*) ...	♂ *u.r.* :		0
,, (*c*) ...	⚥ :		4
,, (*d*) ...	♀ *p.r.* :		11
,, (*e*) ...	♀		40

Table III.*

	A.	B.	C.	No. of times found among records made.
Stage II (*a*) ...	♂	♂ :		4
,, (*b*) ...	♂ *u.r.*	♂ :		1
,, (*c*) ...	⚥	♂ :		3
,, (*d*) ...	♀ *p.r.*	♂ :		12
,, (*e*) ...	♀	♂ :		30
,, (*f*) ...	♀	♂ *u.r.* :		0
,, (*g*) ...	♀	⚥ :		10
,, (*h*) ...	♀	♀ *p.r.* :		5
,, (*i*) ...	♀	♀ :		4

Table IV.*

	A.	B.	C.	D.	No. of times found among records made.
Stage III (*a*)	♂	♂	♂ :		0
,, (*b*)	♂ *u.r.*	♂	♂ :		0
,, (*c*)	⚥	♂	♂ :		0
,, (*d*)	♀ *p.r.*	♂	♂ :		5
,, (*e*)	♀	♂	♂ :		11
,, (*f*)	♀	♂ *u.r.*	♂ :		1
,, (*g*)	♀	⚥	♂ :		8
,, (*h*)	♀	♀ *p.r.*	♂ :		16
,, (*i*)	♀	♀	♂ :		5
,, (*j*)	♀	♀	♂ *u.r.*:		0
,, (*k*)	♀	♀	⚥ :		3
,, (*l*)	♀	♀	♀ *p.r.* :		1
,, (*m*)	♀	♀	♀ :		1

* 1. In these tables the chains read horizontally and the life-histories of the individuals may be read vertically.

2. Some of these chains occur as side-chains.

On examining the whole of the records made of the natural chains, I find that all the conditions of chains shown in Tables II, III, and IV occur except Stage I (*b*), Stage II (*f*), and Stage III (*a*), (*b*), (*c*), and (*j*). The number of times each condition in these stages is found in the whole of the records is put in the right-hand column in Tables II, III, and IV. Probably the earlier conditions of Stage III chains do not occur because the ♂ period of life will already have been passed by "A" individuals before more than one or two males have been able to settle down on them. Since nearly all conditions of Stages I, II, and III are found, I have no doubt that chains are formed as is indicated above. The relative frequencies of different lengths of chains in all the chains examined—including those in Table V—may be gathered from a glance at the curve in fig. 3.

From fig. 3 it is seen that the longer the chains are the less frequently do they occur. Chains of 9, 10, and 11 individuals were not recorded once, and only one chain of 12 individuals was seen.

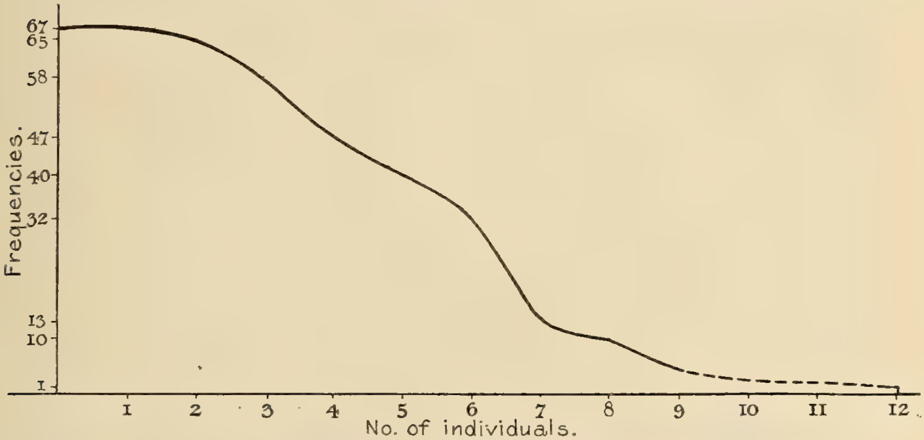

Fig. 3.—Curve showing frequencies of different lengths of chains among 336 chains. Ordinates = frequencies ; abscissæ = individuals in chain.

The age of change from ♂ to ♀, and the length of time required for the formation of a chain of given length are being investigated.

Some points in the records of the chains are noteworthy. The scarcity of ♂ *u.r.*'s is noticeable. Probably their rarity may be accounted for by the following facts.

(1) The uterus develops in the wall of the mantle, and is not visible externally until partly developed:* only those individuals were recorded as ♂ *u.r.*'s in which the end of the uterus projected from the mantle.

(2) The chains have only been examined between February and May this year: it is possible the change may occur rapidly at some other period of the year.

Single chains often afford evidence of the change from ♂ to ♀; such a series as ♀ *p.r.* ♀ *p.r.* ⚥ is common; moreover, in these cases a gradual decrease in size of the penis, from the ⚥ to the proximal ♀ *p.r.* is also often observable; a point not brought out in the records.

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* The development of the uterus is being investigated.

Table V.—Samples of the Records of the Sex Relations in the Chains of *Crepidula fornicata*.

Reference No. of chain.	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.	L.
80	♀	♀	⚥	♂	♂	♂ :						
81	♀	♀	♂	♂	♂ :							
82	♀	♀ :										
83	♀	♀ *p.r.* ...	♂ :									
84	♀	♀	⚥	♂ :								
85	♀	♀	♂	♂ :								
86	♀	⚥ *p.s.* ...	♂	♂ :								
87	*?	♀	♂ :									
88	♀	⚥ :										
89	♀	♀	⚥ *p.s.*									
90	♀	♀	⚥ :									
91	♀	♀	♂ :									
92	♀	♀	♂	♂	♂	♂ :						
93	♀	♀	♀	⚥ *u.s.* ...	♂	♂	♂ :					
94	♀	♀	♀	♂	♂	♂	♂ :					
95	♀	♂ :										
96	♀	♀	⚥	♂	♂	♂	♂ :					
97	♀	♀	♂	♂	♂ :							
98	♀	♀	♂	♂	♂ :							
99	♀	♀	[(♀ *p.r.*)	(A′♂)(B′♂) C′♂ (A″ ♀ *p.r.*) (B″ ⚥ *p.s.*) C″ ♂]								
100	♀	♀	⚥ *u.s.*	♂								
101	♀	♀ *p.r.* ...	♂ :									
102	*?	♀	[(♀ *p.r.*)⚥*p.r.*♂] ...	♀	♀ *p.r.*	[(♀ *p.r.*)(♂)♂]	♀ *p.r.* ...	♀ *p.r.* ...	⚥ *p.s.* ...	♂ ...	♂ ...	♂ :
103	♀	♀	[(♀) ⚥] ...	[(♀ *p.r.*)♂]	♀ *p.r.* ...	♂	♂	♂ :				

320	♂ :
321	♀ :
322	♀ :
323	♀ :
324	♀ *p.r.* :
325	♀ :
326	♀ *p.r.* :
327	♀ *p.r.* :
328	♂ :
329	♂ :
330	♀ *p.r.* :
331	♀ *p.r.* ... ♀ *p.r.* ... ♂ :
332	♀ *p.r.* ... ♀ *p.r.* ... ♀ *p.r.* :
333	♀ *p.r.* ... ♂ ...... ♂ :
334	♀ *p.r.* ... ♂ :
335	♀ ...... ♂ :
336	*? ...... ♀ *p.r.* ... ♀ *p.r.*......... ♀ *p.r.* ... ♀ *p.r.* ... ⚥ *p.s.* ... ♂ ...... ♂ ...... ♂ :
337	♀ *p.r.* ... ♀ *p.r.* ... ♂ .... ♂
338	♀ *p.r.* ... ♀ *p.r.* ... ♂ .... ♂ ...... ♂ :
339	♀ ...... ♀ ...... ♀ *p.r.* [(♀ *p.r.*)(♀)(♀*p.r.*)♂] ♂ ...... ♂ *p.s.* :
340	♀ ...... ♀ ...... ⚥ with eggs ♂ *u.r.* ... ♂ :
341	♀ ...... ♀ ...... ♂ .... ♂ :
342	♀ ...... ♀ *p.r.* ... ♀ *p.r.*......... ⚥ *p.s.* ... ♂ ...... ♂ :
343	♀ ...... ♂ *u.r.* ... ♂ :

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* The question marks indicate that only the shell was present, and that the chains, of which these are the first members, were found unattached to any foreign objects. It is therefore impossible to state whether the occupants of these shells were really the first members of the chains.

Relation of Primary to Secondary Sexual Characters.

Having established the fact that a completely continuous series in the reduction of the penis, and correlated increase in size of the uterus occurs, it became clear that continuity of the primary sexual characters should also be found. Accordingly, an investigation was made with this object in view, by means of serial sections of the gonad.

The gonad in all forms consists of two or three main tubes extending the whole length of the visceral mass, giving off tubular diverticula, which anteriorly divide and subdivide to form a loose compound tubular gland, but which posteriorly divide at most a few times, or are mere blind cæca. The cells lining the tubes proliferate, and some are shed into the lumina as germ cells—eggs or sperms.

In the male the main tubes of the gonad open into a vas deferens, from a dilatation of which a narrow tube leads to a groove on the dorsal surface of the body. This groove runs towards the head from the anterior end of the visceral sac to the base of the penis, and is continuous with a groove in the latter. In the female the main tubes of the gonad open into the oviduct. A receptaculum seminis opening into the oviduct has been described (3). The relation of the gonad to the gonaducts in the ⚥ forms is being investigated. The colour of the gonad in the ♂ is brownish red, that of the ⚥ brownish red or orange, occasionally yellow near the uterus, that of the ♀ yellow.

Sections of the ⚥ forms were naturally cut first, but an examination of the gonad in all those investigated revealed nothing but ova. Sections of the "proximal" males were then prepared, with the result that both ova and sperms were found in the gonad. An examination of the gonad of all the males in a chain indicates, as far as observations go, that there is a gradual increase in ova in the gonad the nearer a male is to the most distal ♀; but even the smallest ♂'s examined have a few ova in their gonad.* It therefore seems doubtful whether pure males, *i.e.* males with only sperm in the gonad, are ever found in *Crepidula fornicata.* Hence the necessity of defining the term "male." The term "male" is usually applied to an organism which produces only sperm in its gonad. As, however, in most of the higher animals a special part of the body is modified into an intromittent organ, the presence of such an organ is adopted as a criterion of maleness. Usually this is a fair inference. In species presenting no sexual dimorphism, such as Amphioxus and Echinoderms, one resorts to the true criterion of maleness or femaleness, namely the production of ova or sperms in the gonad. In *Crepidula fornicata*, therefore, to be strictly accurate one should examine the gonad of every individual possessing even a well developed penis before committing oneself as to its sexual character. As a matter of convenience, however, I have adopted the simpler plan of calling all forms with a penis, males; it being understood that nothing more than the presence of the external male character is implied.

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* Since G. Smith (4) has found ova in the gonad of the ♂'s of many species of Crustacea, it would seem that a careful microscopic examination of the gonads of all males, made in the light of these observations, might bring out important facts bearing on the nature of maleness.

The youngest forms, however, are doubtless exclusively males as regards function, and the oldest forms probably exclusively females.

Examination of the gonad, then, at different periods in the life-history of *Crepidula fornicata,* makes clear that at first it produces ripe sperms only, but becoming with advancing age more and more egg-producing, until finally it is probably entirely egg-producing. There is, therefore, no doubt that all the individuals of this species are born as males, and change in the course of their life-history into females.

It is interesting to note that all stages of the gonad may occur among the individuals of a single chain. A comparison of the primary with the secondary sexual characters made at any phase in the life-history of an individual shows that the development of the former is always in advance of the latter; indeed, the primary sexual characters forecast the secondary sexual characters.*

It has been shown that the right antero-lateral borders of the shells of *all* the individuals in a chain are very close together; since the penis of the male and the external aperture of the uterus of the female are also on the right side anteriorly, it follows that any male in a chain could transfer sperms to any female; but no such transference has yet been observed.

Crepidula fornicata appears to be the only one of the many species of the genus which has taken to the habit of forming chains of more than two individuals;† in several other species, however, namely, *C. adunca, C. plana, C. convexa,* a male is often found mounted on a female (5). *Crepidula fornicata* is also the only species of the genus yet described as hermaphrodite, but probably other species are hermaphrodite, as will be shown below.

In *Crepidula fornicata* it would seem, therefore, that chain-formation and hermaphroditism are in some way causally connected. Knowing, as we do, that most of the genus Crepidula are sedentary in habit, and that sedentariness is associated throughout the whole animal kingdom with hermaphroditism; knowing, further, that a closely allied species, *Crepidula plana,* shows at least signs of, if not complete hermaphroditism (see below), it would seem that chain-formation is an adaptive phenomenon, which has arisen along with, and favoured the acquisition of, protandric hermaphroditism.

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* In view of the conception of a sexual formative substance (4, p. 85), this phenomenon is not without significance.

† See footnote, page 482.

Chain-formation, along with protandric hermaphroditism, effects a strict economy of the sperm of the males, since the sperm is probably all transferred to the females; moreover, this arrangement probably ensures cross-fertilisation of *all* the females. In this respect chain-formation with protandric hermaphroditism is an advantage over permanent hermaphroditism with self-fertilisation, and no doubt leads to as great productiveness as would obtain in a motile unisexual condition. *Crepidula fornicata,* therefore, has become adapted to a sedentary life without losing any of the procreative advantages of a free-living habit.

The individuals forming side-chains are almost always on the left side of the primary chain, and therefore cut off from all sexual relations with any of the individuals of the latter. Here would appear at first sight to be a mal-adaptation, but individuals settling on a primary chain are quite comparable with those settling down on foreign objects; if the phenomenon is not due merely to chance, however, the former would appear to be the more gregarious.

Occurrence of Dwarf Females.

The variation in size in this species is indeed remarkable; it has been well described by Prof. Conklin (5, pp. 438 to 440). Size, besides being dependent on the usual conditions, is also determined by the extent of the surface of attachment. If individuals settle down on a small pebble or other surface where expansion is impossible, they remain permanently dwarfed. Mature dwarfed females have been found in such situations as small as 1·8 cm. long by 1 cm. wide, their shells being generally somewhat thickened, especially around the edge. On a flat surface of unlimited extent individuals may grow to a size of 5·5 cm. long by 3·5 cm. wide. *Crepidula fornicata* is thus able to regulate its shell-forming metabolic processes to the individual requirements. A similar readiness to adapt itself to its situation is exhibited by *C. plana*; but an even greater difference in the size of the extremes has been observed in this species by Prof. Conklin (2, p. 12), a race of dwarfs having been described by him as being one-thirteenth the size of the larger forms!

The smallest females found in *Crepidula fornicata* are the dwarfs mentioned above: they often occur in the middle of an oyster-shell surrounded by chains, the posterior ends of the shells of which converge on the *middle* of the oyster-shell, thus preventing expansion of the enclosed individual. Dwarf females, however, often have ⚥ forms or ♀ *p.r.*'s fixed upon them, and since single settled males are found quite as small, there is no doubt that these small females have once been males and are really dwarfed. These dwarfs, as in the case of those of *C. plana*, appear to be dwarfs merely by reason of their environment, being only, as Prof. Conklin provisionally calls them, "physiological varieties."

In some cases chains were observed in which the proximal individuals were more or less dwarfed, but as the posterior ends of the shells of dwarfs tend to overgrow the surface to which they are attached, especially if such be a small pebble, the dorsal surface of the shell of the proximal individual offers a larger surface than did the pebble, hence the "B" individuals in such chains are able to and do grow bigger than the "A" individuals. In such chains the largest individual is found about the middle, and is often an ⚥ or even a ♂.

Chains dredged up just off the shore at West Mersea were found to consist of larger and more numerous individuals than those dredged up from "the Main," about 20 miles farther down the coast; these, however, may be phenomena arising from the usual conditions which determine size, as food supply, temperature of medium, chemical composition of medium, and so on.

Sex Phenomena in Allied Species.

Prof. Conklin has made estimations of the relative average volume of the ♂'s and ♀'s of several species of *Crepidula*, obtaining the following relations:—

The males of	*C. plana*	are	$\frac{1}{16}$	the size of	the females.
„	*C. adunca*	„	$\frac{1}{8}$	„	„
„	*C. convexa*	„	$\frac{1}{5}$	„	„
„	*C. fornicata*	„	$\frac{1}{4}$	„	„

He therefore naturally concludes that "There is then a marked sexual dimorphism in these molluscs, the mature females being generally much larger than the males; *the females are sedentary, the males locomotive* . . . . ." (2, p. 16).

In another place (5, p. 441) he further states: "In all species of *Crepidula* the males are smaller than the females . . . ." And again (5, p. 442) he states "That in the case of the other species named (*convexa*, *adunca*, *navicelloides*, *plana*) the males are never immovably fixed to one spot . . . , their shells also are not distorted so as to fit irregular surfaces as is the case with the females. In all cases locomotion is limited to small individuals. The young of all species and both sexes crawl about freely and rapidly. In *C. convexa* individuals of both sexes retain this power to a limited extent, *but the large females of adunca, navicelloides, and plana become firmly fixed, whereas the males of these species remain small and retain, to a certain extent, their power of locomotion** . . . . In *C. plana* the shell of the male is more nearly round than that of the female, and is usually more sharp-pointed at the apex . . . . [In a] number of individuals the older part of the shell has the male characters, while the newer part has those of the female.† "In such animals the penis is usually very small, and in some cases has almost entirely disappeared. Quite a complete series of stages in the degeneration of this organ was observed from the fully formed organ on the one hand to a minute papilla on the other. Sections of such animals show that neither male nor female sexual cells are produced at this time (!) The evidence seems to favour the view that we have in these cases an example of protandric hermaphroditism, but I am not able to assert that this is really the case, although I have spent much time in attempting to decide it."

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* The *italics* are mine.

† 2, p. 16.

From these quotations the following facts are brought to light:—

(1) The males in all species of *Crepidula* are smaller on the average than the females.

(2) The females of the species of *adunca, navicelloides,* and *plana* are fixed, but the males are motile.

(3) The adult females and males of *C. convexa* are motile to a limited extent.

In the light of the present observations on *Crepidula fornicata,* I have no hesitation in concluding that *C. plana* is also a protandric hermaphrodite, as Prof. Conklin suspected. It is highly probable also that the species, *adunca* and *navicelloides* are protandric hermaphrodites, but there is not sufficient evidence available for a judgment on *C. convexa.**

A careful research on the proportions of the young males and females, and on the sexual character of the young of the various species of *Crepidula*, may bring out an interesting series of stages in the evolution of protandric hermaphroditism.

Sex Phenomena in the Streptoneura.

It is significant that Pelseneer should remark (6, p. 124) that "sedentary species (of Gastropods) often possess a rudimentary penis." Stimulated by this statement, I examined a collection of 160 *Calyptræa chinensis*, and found that *all* the small ones, about half the number examined, were ♂'s, while the larger ones were either ⚥, ♀ *p.r.*, or ♀, but were nearly all ♀ *p.r.*'s. Since all the small ones are males, however, it would seem that this species is also a protandric hermaphrodite. An investigation is being made of the primary sexual characters to decide the question. It is probable, therefore, that protandric hermaphroditism may be found to be much more common in the Streptoneura than is thought at present. Pelseneer mentions 10 other Streptoneurous hermaphrodites, one of which, *Entoconcha,* is known to be protandric (6, p. 159); three others, *Entocolax, Entosiphon* (7), and *Exteroxenos* (8), are probably protandric. Six others occur, *Valvata, Bathysciadium, Odostomia, Cocculina, Oncidiopsis, Marsenina,* of which I have not found descriptions. *C. fornicata* may now be added to this list. Hence, it would appear that one of the chief distinctions between the Streptoneura and the Euthyneura is beginning to break down.

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* *July* 20.—Since the above was written, Mr. E. Smith has drawn my attention to a chain of *C. navicelloides* (probably ≡ *dilatata*), exhibited in the cases of the British Natural History Museum. I was kindly allowed to examine the Museum collection of Calyptræidæ, and in a collection of *C. dilatata* from Ancud I found the following chain—A, ♀ *p.r.*; B, ♂. In another collection of the same species from Patagonia, out of seven individuals the three smallest were ♂'s, the others being either ♀ *p.r.* or ♀. Thus stronger evidence is adduced for the above statements.

The sex phenomena observed in *Crepidula fornicata* support in a striking manner G. Smith's view (4, pp. 88, 89) of sedentarily-induced hermaphroditism, that is, suppression of females; moreover, the genus may be reasonably expected to offer stages in the evolution of this hermaphroditism, and so afford a means of testing the above-mentioned view. In the early stages of its evolution we should expect to find :—

1. A small percentage of young females among the spat.
2. Adult females of two kinds—
    - (*a*) Those born as females.
    - (*b*) Those born as males.

Recent Researches on Gametogenesis—besides the known fact that some Tænioglossa have two kinds of spermatozoa (6, p. 125)—give some hope that the two latter categories might be distinguishable by the cytological characters of their gametes.

Summary.

Crepidula fornicata is a Streptoneur of the family Calyptræidæ.

Individuals of this species associate permanently in linear series to form "chains." All lengths of chain composed of upwards to as many as 12 individuals have been found.

All the young are able to creep about, but the adults are sedentary.

The individuals in a chain offer a transitional series from maleness to femaleness both in primary and secondary sexual characters. Since all the young ones are males, the species is a protandric hermaphrodite.

Dwarf females occur as "physiological varieties."

Allied species and a species of an allied genus will very likely be shown to be protandric hermaphrodites.

There is good reason for thinking that this sex phenomenon may be even more widely spread in the Streptoneura.

Since the males in this species change into females, it would seem in this case that it is the male which possesses the potentialities of both sexes. A solution to this problem is offered, if, as seems likely, allied species present an evolutionary series in the acquisition of protandric hermaphroditism.

I wish here to express my thanks to the College authorities for the facilities afforded me during the research. I am also deeply indebted to Prof. Dendy and Mr. Darbishire for important suggestions and valuable criticisms.

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(2) Conklin, 'Journ. of Morphology,' vol. 13, 1897, pp. 10—25.
(3) Haller, 'Morphologisches Jahrbuch,' vol. 18, 1892, p. 514.
(4) Smith, 'Fauna and Flora of the Gulf of Naples,' Monograph XXIX, 1906, "Rhizocephala," pp. 91, 92.
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(6) Pelseneer, Lankester's 'Treatise of Zoology,' vol. 5, 1906, 'Gastropoda,' pp. 124, 125.
(7) Koehler and Vaney, 'Revue Suisse de Zoologie,' vol. 11, 1903, p. 36.
(8) Bonnevie, 'Zool. Jahrbüch. Anat. und Ontog.,' vol. 15, 1902, p. 735.

*The Elasticity of Rubber Balloons and Hollow Viscera.**

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(Communicated by Prof. J. N. Langley, F.R.S. Received July 5, 1909.)

(From the Physiological Laboratory, University of Melbourne.)

Introductory Theory.

In an elastic balloon the relation between the internal excess pressure and the tension of the wall can be readily calculated if we assume that the balloon is spherical and that the material is homogeneous and of negligible weight. If we suppose the balloon divided into two hemispheres by a plane horizontal partition, the area of this partition will be πr^2 and the downward force on the upper surface due to the excess pressure p will be $\pi r^2 p$. The balloon wall meets the partition at right angles along a length $2\pi r$. Hence if T is the tension in the wall, the upward force exerted by this tension on the partition is $2\pi r\mathrm{T}$. But as these two forces must be equal we have

$$\pi r^2 p = 2\pi \mathrm{T} r, \quad \text{so that} \quad p = 2\mathrm{T}/r. \tag{1}$$

When such a balloon is filled without stretching the wall the pressure inside is equal to the prevailing atmospheric, and the radius r_0 may be termed the initial radius. If we assume that the balloon is perfectly obedient to Hooke's law, then

$$\mathrm{T}_1 = \mathrm{K}\,(r_1 - r_0)/r_0\,;$$

but from (1) we learn that

$$p_1 = 2\,\mathrm{T}_1/r_1\,;$$

hence, by substitution, $$p_1 = 2\,\mathrm{K}/r_0 - 2\,\mathrm{K}/r_1,$$

or

$$r_1\left(\frac{2\mathrm{K}}{r_0} - p_1\right) = 2\,\mathrm{K}. \tag{2}$$

That is to say, the pressure will increase with radius asymptotically to $2\,\mathrm{K}/r_0$, and if we plot radius against pressure we shall obtain a rectangular hyperbola.

The original object of the following research was to investigate the elastic behaviour of various hollow viscera. Before doing so I decided, however, to carry out a number of experiments with rubber balloons, using the pressures found with varying radii as fundamental data.

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* This research was completed before I became aware, from a reference in Boruttau's 'Lehrbuch der Medizinischen Physik,' that a similar investigation had been carried out by R. du Bois-Reymond, and the results published in the 'Festschrift für Rosenthal.' On obtaining the latter, I found sufficient difference in the treatment of the subject to warrant publication of this. [See also A. Mallock, 'Roy. Soc. Proc.,' vol. 49, p. 458.]

Methods.

The balloons were of the common variety sold as children's toys, and were of varying sizes. A balloon was firmly tied to a glass capillary cannula and held vertically in a glass flask which was immersed up to the neck in an Ostwald thermostat (fig. 1). The temperature of the latter was kept (with a maximum variation of 0°·1 C.) at 35°·5 C. The glass cannula was connected by means of fine-bore pressure-tubing to one limb of a capillary **T**-piece, a second limb of which led to a water manometer, whilst the third limb was connected with a burette for admitting measured volumes of air. The connecting tubes were made as short and of as narrow bore as possible so that the contained volume of air could be neglected in calculation. The burette at its lower end was connected with a levelling tube containing mercury, and at its upper end had a three-way tap. In one position of the tap a sample of air of definite volume and at atmospheric pressure could be taken from the outside air; on the tap being turned this air could be driven through the connecting tubes into the balloon, the mercury being accurately brought to the beginning of the bore of the tap. Conversely, the balloon could be deflated in measured decrements by the same burette. The water manometer consisted of a straight glass tube of 3 mm. bore firmly tied to a vertical scale, and connected at its base with a shorter vertical tube on which was a mark. By means of a three-way tap, capable of connecting the manometer either with the outside air or with an elevated reservoir of water, the level of the water in the shorter limb could be brought to its mark, allowing direct readings to be made from the scale as well as preventing change in the volume of the tube system.

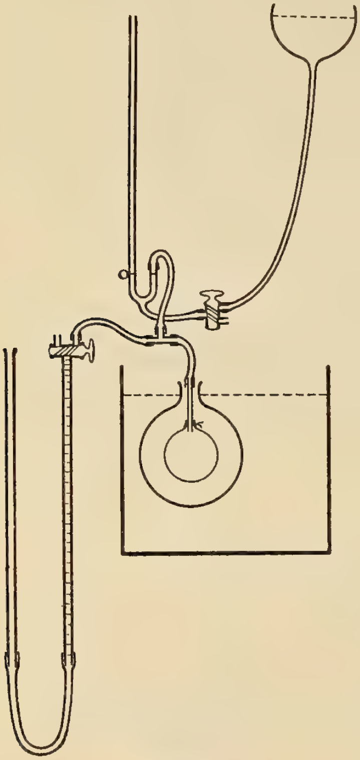

Fig. 1.

The radius of the balloon was calculated from the volume of the air admitted by the usual formula. This involved two assumptions, first that the balloon was spherical, and secondly, that the volume of the enclosed air was the same as that of the air admitted at atmospheric pressure. With the exception of the early stages of inflation and last stages of deflation, when the radius approached the initial value, the balloon could be regarded as a true sphere. As, further, the greatest pressure within the balloon was always a negligible fraction of the prevailing atmospheric, I have not thought it necessary to make any calculated correction as to the volume of the contained air.

When experiments were performed on a hollow viscus, some water was placed in the partially immersed flask, and a few drops placed in the interior of the viscus itself so that the air within and without should be saturated with water vapour.

Experiments on Balloons.

When air was admitted in measured increments to a fresh balloon, and the reading taken a definite time (three minutes) after entrance of each increment, it was found that the pressure rose quickly to a maximum and then on continued inflation fell slowly. This is typically exemplified in the experiment illustrated graphically in fig. 2.

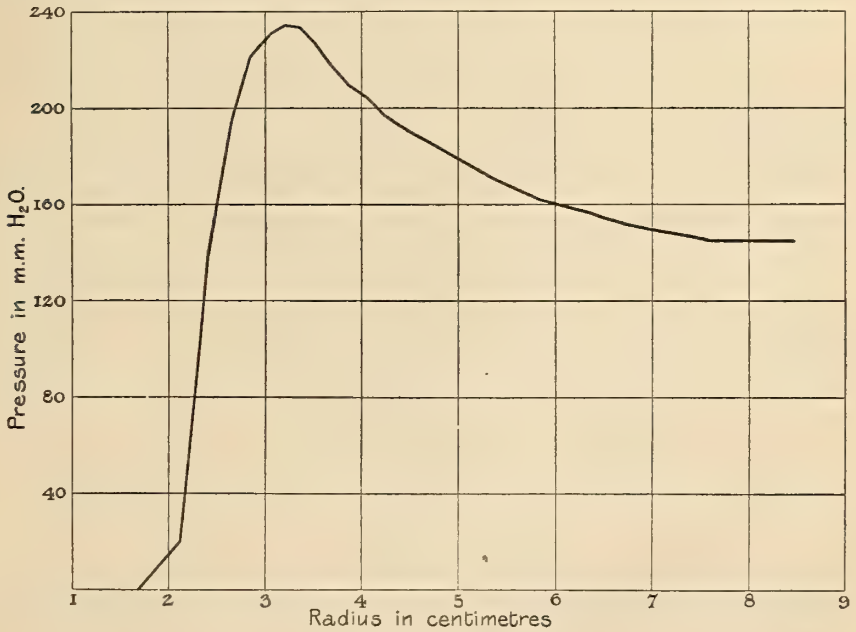

FIG. 2.

It will be seen from this experiment that, over a considerable range, two values of radius can be given for each value of pressure. This can be demonstrated as a class experiment in the following way: Two balloons of equal dimensions are tied to two limbs of a T-tube and inflated by the third limb, which can be closed by a tap. As a rule one of the balloons inflates well, the other remaining small. On closing the tap in the inflating tube the contents of one balloon can be discharged into the other by squeezing with the hand. If the air be worked backwards and forwards a few times to equalise the "history" of each, it will be found that if the balloons are approximately equal in volume they will remain so for a few seconds, in a state of unstable equilibrium, and then one of the balloons will partially deflate itself into the other. The balloon which is now the larger, if squeezed until its volume is slightly less than that of the other and then let go, will continue to deflate until equilibrium is reached.

These experimental results appeared to be utterly at variance with what was deducible from the theory of a perfectly elastic balloon. Amongst the many articles dealing with the elasticity of rubber to which I had access, I found one which promised to throw some light on my results. O. Frank* assumes a somewhat modified Hooke's law. According to him the pressure dP in a sample of section q and length x associated with a shortening dx is given by the formula

$$dP/q = E\,dx/x,$$

in which unit initial length and unit initial cross sectional area are not considered, but length and area such as they are when the change dx is produced. If x_0 is the original length and x_1 the final, he calls $\Lambda = (x_1 - x_0)/x_0$ the specific extension. For the total tension in a strip of unit width and of initial thickness z_0 his final result on p. 608 can be written

$$T = 2\,E\Lambda \frac{z_0}{(1+\Lambda)^2}.$$

Substituting this value of T in equation (1), we get

$$p_1 = \frac{4\,E\Lambda z_0}{r_1(1+\Lambda)^2}.$$

But in the case of the balloon the specific extension $\Lambda = (r_1 - r_0)/r_0$, therefore

$$p_1 = 4\,Ez_0 r_0 \frac{r_1 - r_0}{{r_1}^3}.$$

According to this equation the pressure in an inflating balloon will rise to a maximum when $r_1 = \frac{3}{2}r_0$, and will approach asymptotically to zero when r_1 increases indefinitely. But I may say at once that this approach to zero pressure is never given in balloon experiments, so that Frank's analysis fails to explain the results obtained. One may indeed state *a priori* that as investigations on elasticity are generally confined to substances where the maximum extension is always a small fraction of the initial length, and as Frank's experiments did not follow rubber further than linear extensions to double the initial, it would be almost idle to expect that laws deduced from these experiments could be applicable to the large and two dimensional stretchings of an inflated balloon.

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* 'Annalen der Physik,' vol. 21, p. 602, 1906.

The difficulty in explaining the rise of pressure and the subsequent partial fall on inflation is, I believe, more apparent than real. This crest is due, I take it, to a disturbing factor which, for lack of a better name, may be called initial rigidity. This view is supported by the following facts :—

1. If s fresh balloon is inflated, so that the pressure is anywhere on the rise or fall of the crest, it will be found that the pressure does not remain at a constant value, but tends to fall. In fact, to obtain a graph such as fig. 2, the convention had to be adopted of reading the pressure after a given interval of time—3 minutes. But the fall had by no means stopped when the reading was taken, and could be detected even some hours after inflation. An attempt to register the pressure after a long interval of time when no further fall might be expected, failed owing to the fact that some of the air diffused out, as was proved by deflating the balloon in measured decrements.

2. If a balloon is inflated a second time (care being taken that the elastic limit has not been reached in the first inflation) the crest is always less pointed than in the first inflation. A third inflation gives a more obtuse convexity than the second, and so on. The longer a balloon remains collapsed the steeper is the rise and fall of pressure on inflation. This is particularly marked if the collapsed balloon is exposed to light.

3. When an inflated balloon is deflated in measured decrements and the corresponding pressures recorded, in the vast majority of cases the pressure falls to zero without any rise being manifested. I obtained this pronounced hysteresis constantly in my earlier experiments, and was inclined to look upon it as the invariable behaviour of a balloon during deflation. Fig. 3 gives graphs for two typical instances.

But a rise of pressure may be obtained on deflation if certain conditions are fulfilled. The rubber must be in good condition, the inflation should not be taken far past the maximum pressure, and the return by deflation should be carried out at once. The rise of pressure, however, is never more than a few millimetres of water. The better condition the rubber is in the blunter is the inflation crest and the less abrupt is the deflation fall of pressure. Conversely, the more the rubber has been exposed in a deflated state to light the sharper is the crest and the more abrupt is the deflation fall.

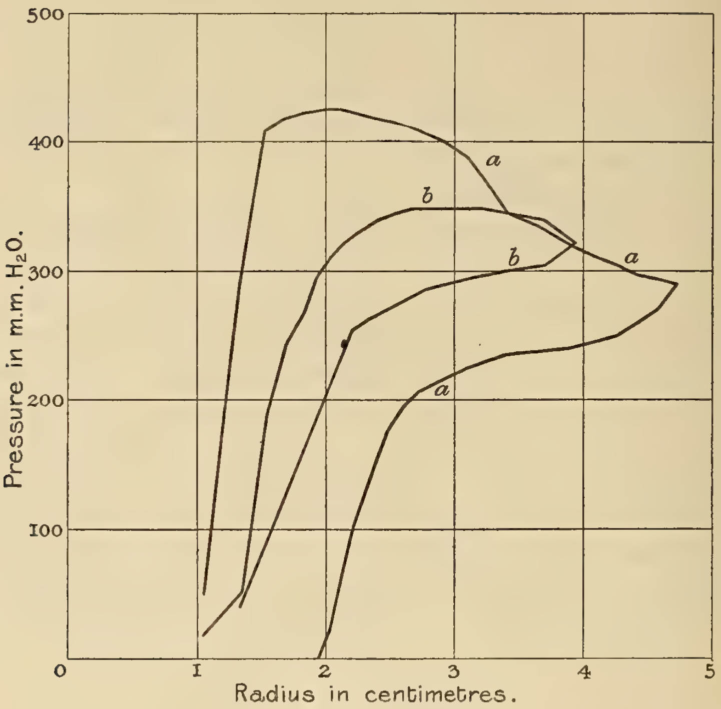

FIG. 3.

I may mention in this connection that if inflation be carried out immediately after deflation the rise of pressure does not follow the same gradient as the deflation fall. It is much steeper, and a crest may be obtained. An illustrative specimen is the following (fig. 4):—

It is easy to demonstrate, however, that the more a balloon is inflated and

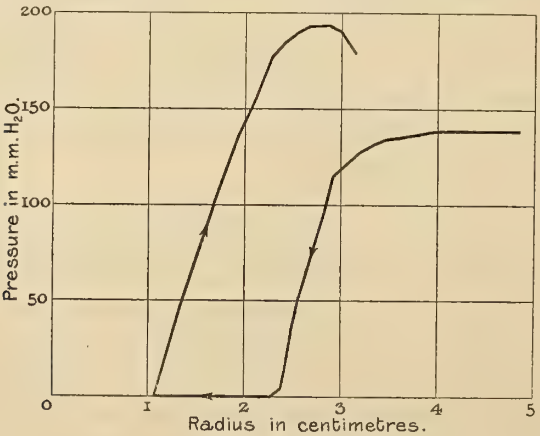

FIG. 4.

deflated, provided that the elastic limit is not approached too closely, the nearer does the inflation pressure gradient approach the deflation. We may regard this as due to the partial removal of the disturbing initial rigidity.

4. If a balloon be inflated until the pressure, after the usual crest, falls and tends to remain constant, and be kept inflated for some time, say 24 hours, and then be rapidly deflated and once more inflated in measured increments, the graph displays no crest and may be a true hyperbolic curve. The following experiment illustrates this important fact:—

A balloon was inflated until the pressure ceased falling, and was kept inflated in the thermostat for 24 hours. It was then rapidly deflated and the usual inflation by the burette commenced. On plotting pressure against radius (fig. 5), I was struck by the regularity of the graph, and recollecting that a balloon of perfect elasticity would give a rectangular hyperbola, proceeded to ascertain if such were the case here. If this were a rectangula hyperbola, the asymptotes being parallel to the co-ordinate axes, it ought to satisfy the equation $(r-a)(p-b) = c$.

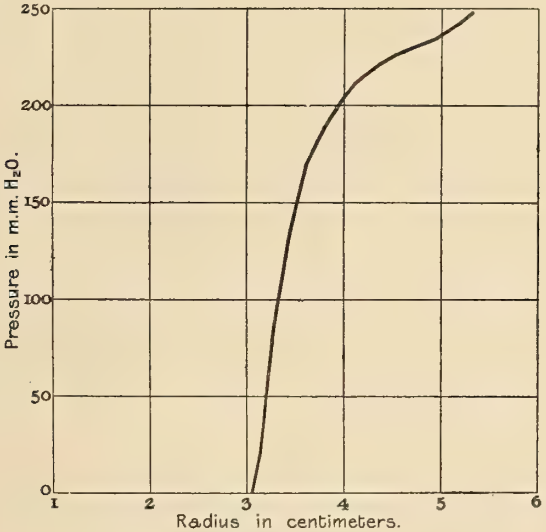

FIG. 5.

To calculate a and b I used the ordinary three-point method. The value for a was found to be 2·8, that of b 287·8.

From radius = 3·29 to radius = 4·37 the product $(r-a)(b-y)$ is a constant. To illustrate this graphically we can plot $b-y$ against the reciprocal of $r-a$, and should obtain a straight line passing through the origin. This is shown in fig. 6.

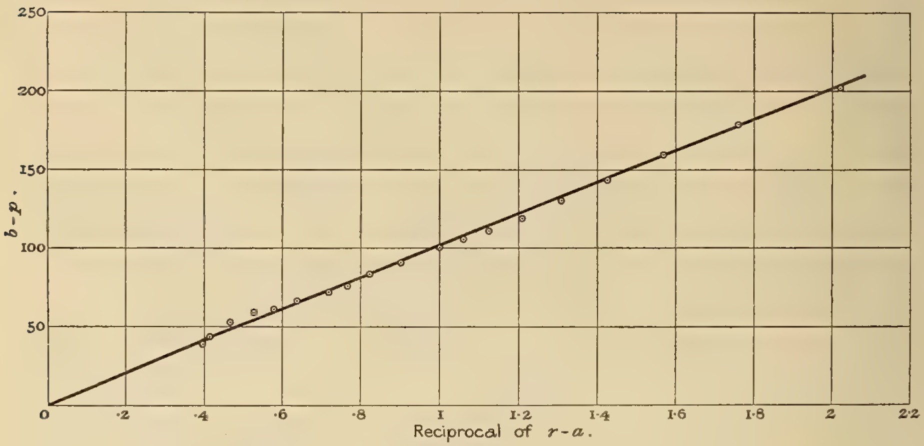

FIG. 6.

As another instance of the applicability of this equation to a balloon, the deflation values already given in fig. 3 may be cited. Calculation by the three-point method gives here $a = 2{\cdot}03$, $b = 263$.

Conclusive as these values are that the rubber balloon, when initial rigidity is removed, follows the equation $(r-a)(p-b) = c$, it will be at once obvious, from the values of a and b found here, that this is certainly not the behaviour of a perfectly elastic substance giving equation (2). For one thing, the value for a is far removed from zero and is suggestively close to that of the initial radius in the two cases investigated. I abandoned the theoretical analysis of my results at this stage, and handed over my data on balloons and on bladders to Mr. William Sutherland, who has kindly complied with my request to comment upon them (see p. 497 below).

Rubber Balloons at the Elastic Limit.

In the course of this research a curious result was obtained with every balloon which I inflated beyond the elastic limit. I invariably found that, before the balloon burst, the pressure, over a considerable range, was a linear function of the volume. Of the many instances obtained I will pick out two, one giving a close approximation to a straight line on plotting volume against pressure (fig. 7).

One of the more divergent types is that given in fig. 8, which is a continuation of the same experiment as fig. 2.

As a rule, the straight line rises abruptly, producing discontinuity in the graph.

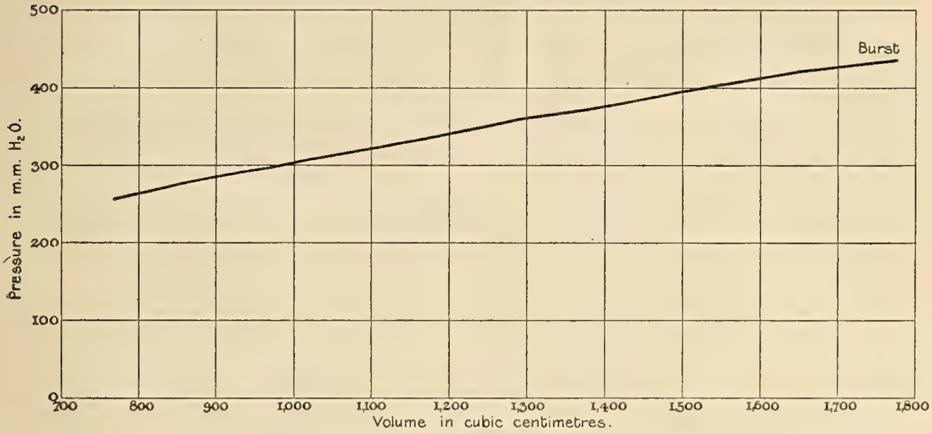

Fig. 7.

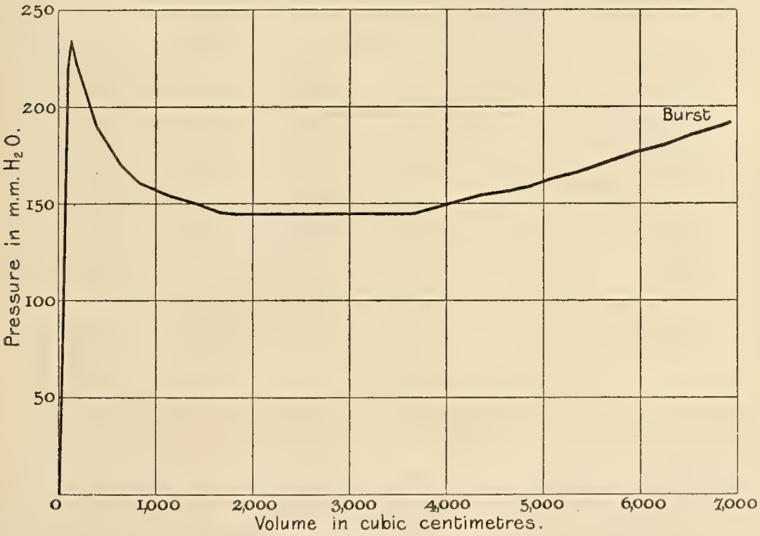

Fig. 8.

Experiments with Hollow Viscera

In these experiments attention was confined chiefly to the bladder, as its shape approaches more closely to the spherical than other viscera. Experiments on lungs proved impossible, owing to the remarkably low bursting pressure of the superficial air cells. A number of observations were made with bladders taken from the recently killed animal, but the erratic behaviour of the living muscular tissue did not allow of a definite pressure being assigned to any stage of inflation. Consistent results could only be obtained by experimenting with bladders some time (24 hours) after the death of the animal.

Fig. 9 shows the results of an experiment with the bladder of a large Newfoundland dog 24 hours after death. As with the balloon, I anticipated that here a hyperbolic curve was present, and calculated by the three-point method the value for *a* to be 0·071, *b* to be 179. Here it will be seen that from radius 1·93 to radius 2·88 a distinct approximation to a rectangular hyperbola is manifest. But even here, though *a* can be made zero without appreciably altering the constancy of *c*, the value for *b* likewise does not allow us to apply to this bladder the formula for a perfectly elastic substance.

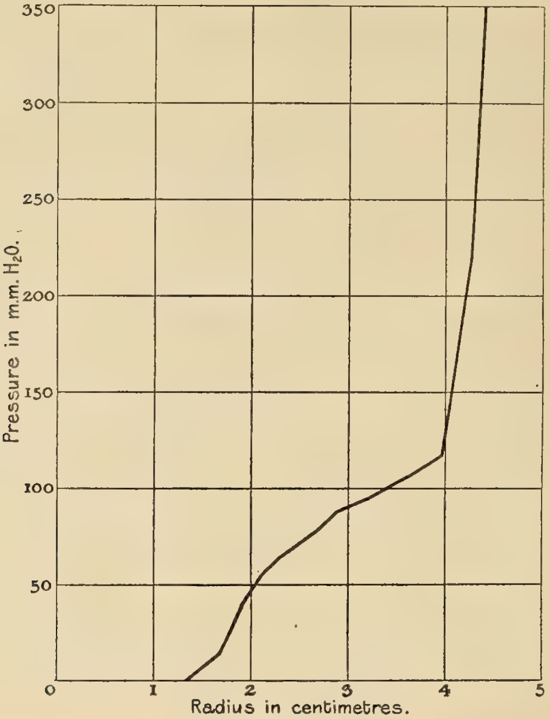

Fig. 9.

A number of bladders of various animals were investigated. I give here the results obtained with the bladders of two monkeys and a cat (fig. 10).

It must be remembered that the elastic tissue of a viscus is not a homogeneous membrane, but a web of elastic fibres with a variable amount of inextensible white fibres intermixed. This fact must always complicate physical investigations on the elasticity of animal membranes, even if the isolated elastic fibres present obeyed some simple physical law.* When we consider the complex æolotropism of a visceral wall, it is indeed surprising that approximations to uniform behaviour, such as are illustrated in fig. 10, should be shown at all.

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* A research on the elastic constants of the *ligamentum nuchæ* is at present being conducted in my laboratory.

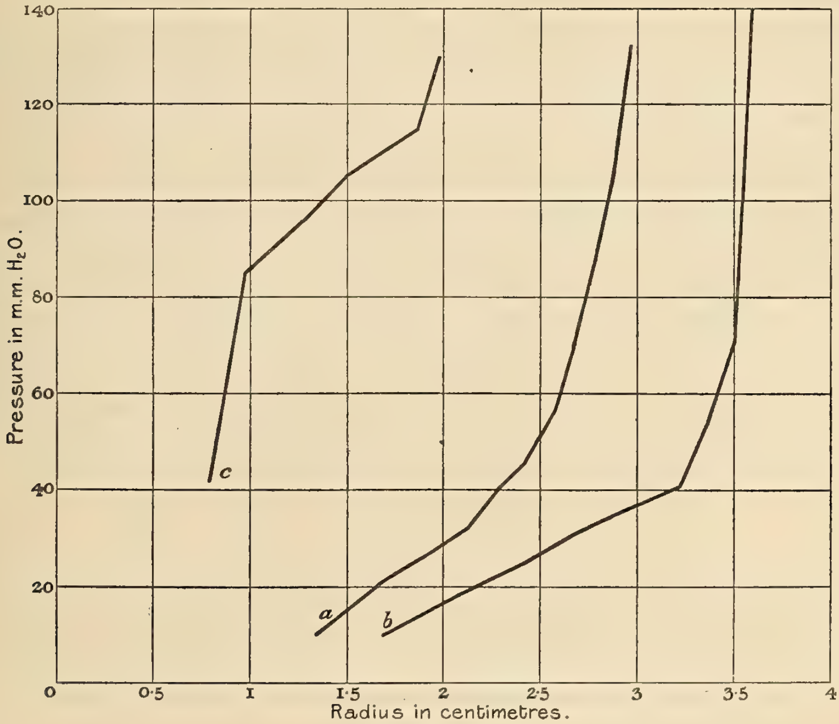

FIG. 10.

R. du Bois-Reymond has conjectured that in hollow viscera the pressure may fall with increasing volume. I may state at once that I have never found this. What sometimes does happen (and to this Du Bois-Reymond's statement is possibly due) is that, on extensive inflation, one of the coats of the organ may give way and lead to a marked drop in pressure. The suddenness of the drop will always indicate the true nature of the fall, and if the organ be now deflated and then inflated again, a consistent rise of pressure will be obtained. Moreover, as I have endeavoured to show, a fall of pressure on continued inflation is only found in balloons manifesting initial rigidity, and such initial rigidity is altogether absent from animal membranes kept moist.

A bladder always displays some hysteresis on deflation, but I have found that this hysteresis can be made negligibly small—(1) if the elastic limit is not approached too closely; (2) if the inflation and deflation are carried out by very small increments and decrements respectively; and (3) if on deflation some time be allowed to elapse at each stage before reading the pressure, as this always tends to rise somewhat. When the elastic limit of a bladder is reached, the gradient of the pressure rise is very steep and the rise is not a linear function of the volume.

There is always a danger that in investigations on elasticity one may forget that the viscus in question in the living animal is supplied with reactive muscle, and that only when this muscle is fully inhibited can the pure physical elasticity of the walls play a predominating part. It is a mistake to describe the flow of blood in the systemic arteries as a flow of liquid in elastic tubes. Such is certainly the case in the aorta, and possibly in the larger arteries, but in the arterioles and smaller arteries only when the muscle is fully inhibited or killed. To describe the circulation as occurring through a system of muscular tubes, with some elastic tissue aiding the muscles, would be more accurate. Similarly with the bladder and other hollow viscera (except the lung), the elastic tissue acts merely as an adjuvant to the muscle, economising the work of the latter; but it is the muscle which plays the preponderating part in determining the tension of the visceral wall.

Conclusions.

1. When initial rigidity is present in a rubber balloon, the pressure on inflation rises rapidly at first, then falls, and tends to remain at a constant value until the elastic limit is reached.

2. Such a balloon on deflation displays a marked hysteresis. Only rarely will the pressure rise on deflation.

3. If initial rigidity be abolished by keeping a balloon inflated some time and then rapidly deflating, the pressure on a new inflation rises consistently. On plotting pressure against radius in such cases a rectangular hyperbola may be obtained, satisfying the equation

$$(r-a)(p-b) = c,$$

where a is close in order of magnitude to the initial radius, and b is a constant greater than p. The behaviour of such a balloon is, however, far removed from that of a sphere of perfectly elastic and isotropic material.

4. When the elastic limit is reached in a rubber balloon the pressure is a linear function of the volume.

5. Hollow viscera approximately spherical, such as the bladder, do not display initial rigidity, and never give a fall of pressure with increasing volume. When the elastic limit is reached, the pressure is not a linear function of the volume.

6. In the bladder of a large dog, giving sufficient range between the assumption of globular form and the elastic limit to allow analysis of the graph of pressure against radius, it was found that the equation

$$(r-a)(p-b)=c$$

was followed. In this case a was practically zero; but like the rubber balloon the behaviour was not that of a perfectly elastic and isotropic substance.

Note on the foregoing Paper by W. SUTHERLAND.

From the purely physical point of view the simplest way to prepare for a theoretical interpretation of experiments such as these is to fix attention in the first instance on tension per unit area.

Let the tension per cm.^{2} be t in the balloon or bladder which has radius r and thickness z. Let initial values of these, when $t=0$, be r_0 and z_0. Consider the equilibrium of a hemisphere. It experiences a pull $2\pi rzt$ from the other hemisphere. But on account of the excess p of the pressure inside the sphere over that outside the hemisphere is subject to a thrust $\pi r^2 p$; thus

$$\pi r^2 p = 2\pi rzt \quad \text{or} \quad pr^2 = 2rzt. \tag{1}$$

If, as in studying the surface tension of bubbles, we fix attention on zt, the total tension across unit width of cross-section of the bounding wall, and call it T, we have

$$p = 2\mathrm{T}/r. \tag{2}$$

According to Hooke's law, we write

$$t = \mathrm{E}(r-r_0)/r_0, \tag{3}$$

where E is a modulus of elasticity appropriate to the conditions of the experiment, which in the present case are equal tensions in two dimensions and no external stress in the third dimension. For substances such as rubber and most organic tissues which have a compressibility, small in comparison with their deformability, E for small strains is twice the ordinary Young's modulus for small strains. But when large strains are used, as in these experiments, E can no longer be treated as a constant. It is a function of the strain. This appears when we compare Prof. Osborne's formula $(p-b)(r-a)=c$ with (1) and (3), after elimination of z by the relation $r^2z = r_0^2 z_0$ expressing incompressibility, for we get

$$p = \frac{b(r-a)+c}{r-a} = \frac{2\mathrm{E}z_0(r-r_0)r_0}{r^3}. \tag{4}$$

This makes E a complicated function of r.

In the experiments with the dog's bladder, a is nearly 0, so that this takes the simpler form $E = \frac{r^2(br+c)}{2z_0(r-r_0)r_0}$, which is still too awkward for interpretation. But to connect the results for the tissue of dog's bladder with those for other tissues the modulus of elasticity E can be regarded from a different point of view. In experiments on dead muscle, for instance, the muscle is stretched by different weights, the amount of stretching produced by each being recorded. As the muscle is lengthened its cross-section is diminished, but, as a rule, no account is taken of this fact. This is because more interest is taken in the behaviour of the muscle as a whole, or of a single representative muscle fibre, than in the intensity of the tension or the tension per cm.^{2} of cross-section of the muscle. For the gastrocnemius of the frog stretched by amounts $l-l_0$, by weights w up to 95 grammes, C. Henry has shown* that the following formula holds:

$$l-l_0 = 6{\cdot}55 \log(1+w/6{\cdot}10), \tag{5}$$

$l-l_0$ being expressed in mm. and w in grammes weight. For other tissues with a wide range of elastic properties, A. Goy† finds the same formula to apply with appropriate values in place of 6·55 and 6·10. But the physical explanation given for (5) by Henry is not sound, as he interprets $1+w/6{\cdot}10$ in the form $(6{\cdot}10+w)/6{\cdot}10$ to mean that there is at the beginning a tonus of the muscle equivalent to a weight 6·1 grammes. If there is stress in the muscle at the beginning it must be self-equilibrating, and it is not correct mechanics to fix upon one part of this internal stress, called the tonus, and treat it as a sign of a not otherwise demonstrable external force denoted above by 6·1. But, guided by the success of (5), we can arrive at a simpler formula which is capable of legitimate and easy physical explanation. Let us suppose that the elongation $l-l_0$ caused by w is related to w by the following equation:

$$(l-l_0)/w = a-b(l-l_0), \tag{6}$$

where a and b are constants for a given tissue.

This means that the average elongation caused by unit weight, that is to say $(l-l_0)/w$, diminishes with increasing w in such a way that the diminution is linear in the total elongation produced by w. When $b = 0$, we have the usual Hooke's law for small strains. It is possible to give a theoretical molecular explanation of (6), though it would not be appropriate here. In the case of the frog's gastrocnemius, for values of w from 30 to 95 grammes, it gives the elongation $l-l_0$, with a maximum error of 1·6 per cent., and from 0 to 30 grammes with a maximum error of 16 per cent., the corresponding error for (5) being 17 per cent. But it is probable that in neither case are these really errors of formula, because with the smaller weights there is liability to considerable experimental uncertainty while "taking up the slack" of the specimen.

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* 'Compt. Rend.,' vol. 162, 1906, p. 729.

† *Ibid.*, p. 1158.

It is interesting to see how the type of formula (6) applies to Prof. Osborne's experiments on the bladder of a dog. We must treat the experimental facts so that they are as similar as possible to those of muscle. If we return to (1) we see that $\pi p r^2$ corresponds with w the weight used to stretch muscle, although it stretches the bladder wall in two directions at right angles to one another. The chief effect of this stretching in two directions is to replace E as measured on a strip cut from the bladder wall and stretched only in one direction by 2 E. From the experiments we get

$$\frac{r-r_0}{\pi p r^2} = 0{\cdot}00121 - 0{\cdot}000318\,(r-r_0), \tag{7}$$

with the following comparison:—

r	1·336	1·680	1·927	2·121	2·285	2·429	2·672	2·878
p exper. ...	0	14	40	55	63	68	77	87
p calc.	0	35	50	58	64	68	76	82

r	3·220	3·637	3·959	4·252	4·493	4·655	4·856
p exper. ...	95	107	117	217	410	560	620
p calc.	95	116	142	182	243	318	533

At the two lowest pressures after 0 the discrepancy between calculation and experiment is large, but can plainly be ascribed to the taking up of slack in the experiments. The formula fits the facts satisfactorily over the very great elongation from $r = 2{\cdot}121$ to $r = 3{\cdot}220$. Beyond that the formula ceases to give the connection between p and r in a useful manner, but on that account it by no means loses its physical significance. If we write (7) in the form

$$p = (r-r_0)/\pi r^2 \{0{\cdot}00121 - 0{\cdot}000318\,(r-r_0)\}, \tag{8}$$

we see that for values of $r-r_0$ greater than 3 the difference $0{\cdot}00121 - 0{\cdot}000318\,(r-r_0)$ becomes small compared with either 0·00121 or $0{\cdot}000318\,(r-r_0)$. Hence a small error in r produces a much larger relative error in p. With this fact in view it appears that (7) gives a good account of the physical happenings in the wall of the bladder during the large elongations up to $r = 4{\cdot}856$.

The form (7) can be applied to the experiments on a deflated rubber balloon, but not to those on an inflated.

The Modes of Division of Spirochæta recurrentis *and* S. duttoni *as observed in the Living Organisms.*

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(Communicated by Prof. G. H. F. Nuttall, F.R.S. Received July 26, 1909.)

(From the Quick Laboratory, Cambridge.)

The exact mode of division of Spirochætes is still a matter of controversy. Some workers, as Prowazek, and most of the German school of protozoologists, consider that Spirochætes divide longitudinally, while Novy, Swellengrebel, Laveran and Mesnil, and most French workers consider that they divide transversely. Unfortunately, the direction of division of Spirochætes has been made a criterion of their protozoal or bacterial nature. Too much stress appears, from this point of view, to have been laid on the mode of division in Spirochætes. Fantham (June, 1907, and January, 1908), in the case of *Spirochæta balbianii* and *S. anodontæ*, and Dutton and Todd (November, 1907), in the case of *S. duttoni*, state that both longitudinal and transverse divisions occur, while Breinl (November, 1907) figured (but did not describe in detail) both modes of division in *S. duttoni*. Fantham worked on both living and stained material. Miss Mackinnon (1909), working in the Quick Laboratory, has recently shown that both modes of division occur in *S. recurrentis*. We hope to show in this paper, from observations on the living organisms, that both methods of division certainly do occur in Spirochætes; also how these processes are brought about, and to put forward suggestions explaining the phenomena.

The subject is one of great difficulty, and the intrinsic difficulty has not been lightened by the methods adopted by various investigators. Too much reliance has been placed on the examination of fixed and stained preparations of Spirochætes, to the exclusion of evidence which might have been derived from observation of the living organisms. It is only recently that the importance of observations of living material in protistology has been recognised, and even now it is not sufficiently appreciated.

During the past year we have observed living *S. recurrentis* and *S. duttoni* at various times. The investigations were conducted at first independently (those on *S. duttoni* by A. P., and those on *S. recurrentis* by H. B. F.); afterwards we worked in collaboration and carefully checked and correlated each other's observations. As a result, we believe that we have arrived at a solution of the difficult problem of the mode of division of Spirochætes.

Material and Methods.

The blood of tame mice infected with *Spirochæta duttoni* and *S. recurrentis* (Russian variety) was used as the source of the Spirochætes. The preparations of freshly drawn peripheral blood were transferred at once to the stage of a microscope enclosed in a thermostat kept at blood temperature (37° C.). In this way the Spirochætes could be observed for many hours.

At other times, for purposes of comparison, no thermostat was used, and the preparations were examined at room temperature. Freshly drawn blood from the heart, liver, spleen, and kidneys of infected mice was also examined. The objectives used were Zeiss' 2 mm. apochromatic homogeneous immersion, or Zeiss' $\frac{1}{12}''$ achromatic, with compensating ocular 8.

Careful observations were also made on living *Spirochæta anodontæ*, in order to determine whether there is a uniformity in the methods of division in the Spirochætes of mammalian blood, and in the Spirochætes occurring in the crystalline styles of Lamellibranchs. Thin portions of infected styles from *Anodonta cygnea* were examined, the preparations being kept at the temperature of the room. Other portions of infected styles were allowed to dissolve in water, and preparations were made. Living specimens of *S. anodontæ* were under observation for as long as 50 hours.

Longitudinal Division.

Longitudinal division of *S. duttoni* and *S. recurrentis* is best seen when the blood under examination contains relatively few Spirochætes. We have found such to be the case at the onset of the infection. At this time there are but few Spirochætes in the blood, and their movements can be followed, though it is at all times a difficult task. As the number of Spirochætes during the early stages of infection is few, the danger of confusing longitudinal division with entanglement figures is at a minimum. This possible source of error has been carefully considered and eliminated.

The Spirochætes about to divide longitudinally are slightly thicker than the other forms, and are somewhat slower in their movements. At the onset of division, waves resembling the peristaltic waves seen travelling along the intestine of an insect begin to pass down the body of the Spirochæte. These waves were carefully differentiated from the spirals of the body of the parasite. A split appears at one end of the organism, and two distinct free ends are seen. The undulations continue to pass down the body, each free part having its waves vibrating in unison with the other. As the length of the free portions gradually increases, they diverge from one another, and their appearance resembles that of the arms of a **Y**. The divergence continues until, at the actual moment of separation, the two daughter organisms are practically in a straight line, that is, there is an angle of 180° between them. When the two individuals are quite free of one another, they usually remain quiescent for a few seconds, and then move off in different directions.

Both *S. duttoni* and *S. recurrentis* divide longitudinally after the manner outlined above. We have repeatedly seen this occur in life in both these Spirochætes, and also in *S. balbianii* and *S. anodontæ*. The time required for complete division is variable. The complete longitudinal division of *S. recurrentis* has taken ten minutes, that of *S. duttoni* thirteen minutes, but much variation was shown by different specimens.

Transverse Division.

Transverse division of Spirochætes of the blood has been definitely stated to occur by various workers, but few of them state precisely what use was made of living and of stained material respectively. Naturally, therefore, details of the exact processes of transverse division are wanting. We have made a careful study of the behaviour of living *S. duttoni*, *S. recurrentis*, and *S. anodontæ* during transverse division, and under conditions resembling, as far as possible, those that obtain in the host.

Transverse division occurs in very long, thin individuals, whose movements are somewhat slower than those of other Spirochætes in their vicinity. The division is initiated by the appearance of waves passing from each end of the organism towards its centre. When the waves reach some particular spot at or near the centre that serves as a node, they meet and die out, return waves passing rapidly in the opposite directions towards either end. A second set of waves then passes from the ends in the direction of the centre; these meet and die out, and return waves, travelling in opposite directions proceed to each end of the organism. These processes are repeated many times, the velocity of the waves increasing meanwhile. As time goes on, the nodal region of the organism becomes thinner, and finally, after several extremely rapid sets of waves in succession, the tenuous centre parts, and two complete short organisms are produced. The parent organism appears to increase slightly in length during transverse division. The individuals resulting from the division usually swim away in the ordinary manner.

The time needed for complete transverse division varies with the individual Spirochæte, but we have observed specimens of both *S. duttoni* and *S. recurrentis* that took from fourteen to sixteen minutes for complete transverse division.

The processes of transverse division, as seen in the larger *S. anodontæ*, exhibit yet further details. One of us (H. B. F.) has already shown that Spirochætes move by (α) an undulatory flexion of the body for forward progression, and (β) a corkscrew motion of the body as a whole. It may be of interest to note that in the transverse division of *S. anodontæ*, as observed in the living organism, reversal of the direction of the corkscrew or helicoid motion of the parasite may occur. In a specimen about to divide transversely, each of the halves vibrating about a node may reverse its direction of torsional movement; for example, a right-handed spiral may suddenly become a left-handed one and *vice versâ*. The resultant strain at the node probably aids in the actual transverse division.

General Remarks.

The fact that both longitudinal and transverse division take place explains the occurrence of thick and thin and of long and short forms of the same species of Spirochæte. Everyone who has worked on Spirochætes is cognisant of the occurrence of such polymorphism.

Longitudinal division of *S. recurrentis* and *S. duttoni*, as before mentioned, is best seen when the blood contains relatively few Spirochætes—at the beginning of infection and also at the end. Breinl (1907) noted the occurrence of longitudinal division "especially at the time of the disappearance of the parasites from the peripheral circulation." Numerous long tenuous Spirochætes are present in the blood during the height of the infection, and these divide transversely. While allowing for the reactions of the host upon the parasites, which reactions might induce the different forms of division at different periods, it is also possible that the *number* of Spirochætes present in the blood at any given period may have some effect on the direction of their division. The greater space necessary for longitudinal division occurs at the onset of infection, when the Spirochætes are few. As the parasites grow in length and increase in numbers, it would appear easier for transverse division to take place.

We have, then, clearly shown that there is a distinct *periodicity* in the direction of division exhibited by *S. recurrentis* and *S. duttoni*. Naturally there is a time when both forms of division go on side by side. The conflicting statements regarding the direction of division of Spirochætes are thus explained and reconciled.

From the foregoing it is clear that the direction of division of Spirochætes cannot be used alone as a criterion of their protozoal or bacterial nature.

The Spirochætes exhibit characteristics of both Protozoa and Bacteria. The reasons for considering them as Protozoa have been well set forth by Nuttall (1908), while Swellengrebel (1907) considers them to be Bacteria, belonging to the family *Spirillaceæ.* On the whole, we consider that the protozoal characteristics of Spirochætes preponderate over their bacterial characteristics.

We have much pleasure in thanking Prof. Nuttall for the material used in these researches.

Summary.

1. The observations recorded in this paper were made on living Spirochætes. We have previously examined much fixed and stained material. It is very necessary to examine living material, as results based only on stained preparations are not always reliable.

2. Both longitudinal and transverse division occur in Spirochætes as seen in *S. recurrentis, S. duttoni, S. anodontæ,* and *S. balbianii.*

3. Longitudinal division of *S. recurrentis* and *S. duttoni* is best seen when there are but few Spirochætes in the blood. This is the case at the onset of infection and at its close. In longitudinal division, rapid waves pass down the body of the Spirochæte. At one end a split occurs, which gradually widens. Waves travel down each of the diverging daughter forms, which ultimately lie at an angle of 180° with one another. The daughter Spirochætes then separate. Organisms about to divide longitudinally are slightly stouter than the others.

4. Transverse division of *S. recurrentis* and *S. duttoni* also occurs. It is initiated by the appearance of waves passing from both ends towards the centre of the organism (which centre acts as a node). These waves meet and die out, and return waves pass rapidly from the centre towards each end. These processes are repeated many times, the frequency of the waves increasing and the nodal region becoming thinner. Finally, after a succession of very rapid waves, division occurs at the node and two complete daughter organisms result.

5. There is a periodicity in the direction of the division of *S. recurrentis* and *S. duttoni.* At the onset of infection, longitudinal division occurs. This is followed by transverse division of the Spirochætes when the infection is at its height, while, with the diminution in numbers of the parasite as the infection draws to an end, there is a reappearance of longitudinal division. Naturally, there are times when both forms of division occur together. Our observations relating to periodicity were made on peripheral blood of the host.

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The Origin and Destiny of Cholesterol in the Animal Organism. Part VI.—*The Excretion of Cholesterol by the Cat.*

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(Communicated by Dr. A. D. Waller, F.R.S. Received August 14, 1909.)

(From the Physiological Laboratory, South Kensington, University of London.)

In an earlier paper* of this series the results of a number of estimations of the cholesterol content of the fæces of a dog fed on a variety of diets—animal and vegetable—were described. It was shown that the cholesterol found in the case of meat diets could be entirely accounted for by that present in the food, and from a general survey of the whole of the results, the opinion was expressed that the whole of the cholesterol of the bile is not excreted in the fæces, and must therefore have been either totally destroyed or reabsorbed in the gut along with the bile salts. In the case of a diet of raw brain, it was found that the cholesterol was not excreted as such, but entirely in the form of coprosterol. This was subsequently† found to be the case when cats were fed on either raw or cooked brain.

* 'Roy. Soc. Proc.,' B, vol. 80, 1908.

† 'Roy. Soc. Proc.,' B, vol. 81, 1909.

A few months after the appearance of our paper, Chosaburō Kusumoto* published a series of estimations of the cholesterol content of the fæces of dogs fed on horseflesh, and horseflesh with the addition of measured quantities of fat bacon or carbohydrates. His results showed that the cholesterol content of the food was considerably greater than that of the fæces. He also found that on meat diets variable quantities of coprosterol were always excreted with the cholesterol, and that the proportion of coprosterol increased with the fat in the food. This explains our observations on brain diets, the putrefactive changes which cause the formation of coprosterol being favoured by the presence of fats. The daily outputs of cholesterol observed by Kusumoto are somewhat greater than in the case of the dog we used, but his daily rations were much larger. He gives no data to indicate the purity of the specimens of cholesterol weighed. As dogs are omnivorous feeders, it seemed desirable to examine the fæces of more truly carnivorous beasts, and for this purpose the cat was selected. The fæces collected during each diet period were dried in the water oven, roughly powdered, or, if too greasy, ground with plaster of Paris, and extracted thoroughly for many days in a Soxhlet's apparatus with ether. The ethereal extracts were treated in the manner fully described in our paper† on "The Cholesterol Contents of Eggs and Chicks."

Experiments in which cats were fed on meat diet:—

I. A cat, which had previously been fed on raw brain diet, for the purpose of another experiment, for 14 days, during which it went down in weight from 2·8 to 2·3 kilogrammes, was fed for 14 days on a diet of lean cooked horseflesh. During this period it devoured 3125 grammes of the meat, and increased in weight steadily, its weights taken every third day being 2·3, 2·5, 2·5, 2·7, and 2·9 kilogrammes. The total dried fæces weighed 98 grammes, and yielded after the treatment described 2·554 grammes of unsaponifiable matter in the form of an oily mass. This was crystallised from alcohol repeatedly, but only 0·2052 gramme of pure crystalline matter was obtained. This melted at 93°—99° C. and had the characteristic crystalline form of coprosterol. The mother liquors were evaporated to dryness and treated in pyridine solution with excess of benzoyl chloride. On pouring into water the benzoate of coprosterol was thrown out of solution, and on washing with a small quantity of alcohol was sufficiently pure for weighing. The total weight of coprosterol obtained was 1·397 grammes, which corresponds to an output of 0·098 gramme per day. If we take Dormeyer's* figure, 0·23, as the percentage of cholesterol in dry muscle, and assume that the cooked meat contained 62 per cent. of moisture,† the animal should have received in its food some 2·7 grammes of cholesterol. A considerable quantity of cholesterol, therefore, must have been absorbed by the animal, which was putting on flesh during the whole experiment.

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* "Über den Cholesterolgehalt der Hundfäces bei gewöhnlicher Ernährung und nach Fütterung von Cholesterin," 'Biochemische Zeitschrift,' vol. 14, 1908, pp. 411 and 416.

† 'Roy. Soc. Proc.,' B, vol. 81, 1909, p. 129.

II. A cat, which had previously been fed on a diet of cooked brain for 14 days, during which it lost weight from 2·9 to 2·5 kilogrammes, was fed for 14 days on lean cooked horseflesh. During this period it devoured 2940 grammes of the meat, and its weights taken every other day were 2·5, 2·5, 2·7, 2·8, 2·9, and 2·8 kilogrammes. The total weight of dried fæces was 92 grammes, which gave 3·02 grammes of unsaponifiable matter. On repeated crystallisation from alcohol, 0·588 gramme of pure coprosterol, melting at 93°—98° C., was obtained. The mother liquor on benzoylation yielded a further quantity of coprosterol benzoate. The total coprosterol thus obtained weighed 1·077 grammes, corresponding to an output of 0·077 gramme per day. Calculating as before, the animal received in its food 2·56 grammes of cholesterol.

III. In this experiment a healthy cat was fed for 14 days on 1550 grammes of lean cooked meat—beef and mutton. Its weight remained practically constant during the experiment. 44 grammes of dry fæces were obtained, which yielded 1·331 grammes unsaponifiable matter. On treatment this gave 0·27 gramme of white crystalline matter, melting at 129°—132°, which appeared to be a mixture of cholesterol and coprosterol. A further quantity as benzoate was isolated from the mother liquors. Total weight of cholesterol and coprosterol, 0·5486, corresponding to an output of 0·032 gramme per day. Calculating as before, the animal received in its food 1·35 grammes of cholesterol.

IV. As the experiments described are possibly open to the criticism that the sameness of the diet over a long period may have affected the metabolism, in this experiment we took four cats and fed them for *seven* days on a diet of raw lean bullock's heart. The animals appreciated this food and took it greedily. They consumed in seven days 5166 grammes, the daily ration of each animal being of the same weight. On the eighth day each animal had a meal of cooked wheat germ, which had previously been freed from fat and phytosterol by extraction with ether, in order to sweep out the gut. The fæces were very oily in character and difficult to dry at

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* 'Pflüg. Archiv,' 1896, vol. 65, p. 99.

† A sample of this cooked horseflesh was dried at 100° C. and found to contain 62 per cent. of moisture.

80° C. The weight of partially dry stuff was 362 grammes. The weights of the animals were taken at the beginning of the experiment and periodically afterwards with the following result:—

	At beginning.		On third day.		On last day.	
	lbs.	ozs.	lbs.	ozs.	lbs.	ozs.
Cat I	8	2	7	14	7	12
,, II	7	2	6	14	7	2
,, III	6	2	6	1	5	5
,, IV	5	4	5	4	5	6

The fæces were extracted in a Soxhlet's apparatus with ether for 19 days and yielded 4·3985 grammes of unsaponifiable matter. This was a dark stiff vaseline-like substance. The unsaponifiable matter was repeatedly crystallised from alcohol, but it proved exceedingly difficult to purify. Eventually 0·05 gramme pure coprosterol, melting 99°—102°, was obtained. The impure crystalline crops and all the mother liquors, after evaporating to dryness, were separately treated in pyridine solution with excess of benzoyl chloride. On pouring into water the crude benzoates which separated were treated in a similar manner to that described in a former paper.* 1·7272 grammes of benzoate was obtained. This melted at 125°—128° without showing any play of colours. It appeared likely that we were dealing with a mixture of cholesterol and coprosterol benzoates, and the substance was, therefore, fractionally crystallised from ethyl acetate. The first crop of crystals on heating began to soften at 130° C. and melted to a turbid liquid at 141° C., which became clear at 165° C. On cooling, the play of colours characteristic of cholesterol benzoate was shown in a well-marked manner. A microscopic examination showed that it consisted of the characteristic plates of cholesterol benzoate with a comparatively small quantity of coprosterol benzoate. A later crop, which a microscopic examination showed to consist mainly of coprosterol benzoate with only a very few crystals of cholesterol benzoate, melted at 117°—119° to a clear liquid. Pure coprosterol benzoate melts at 120°—121° C. Evidently, therefore, a mixture of cholesterol and coprosterol was excreted, the total weight being 1·4004 grammes, corresponding to an output per day per cat of 0·05 gramme.

Heart muscle contains between 0·066 and 0·071 per cent. of cholesterol,† so that the animals received in their food about 3·5 grammes of cholesterol.

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* "Origin and Destiny of Cholesterol in the Animal Organism," Part IV, 'Roy. Soc. Proc.,' B, vol. 81, p. 129.

† "Cholesterol Content of Heart Muscle," 'Journ. Physiol.,' vol. 38, 1908, 'Proc.,' p. 1.

The results of these experiments are summarised in the following table:—

Experiment.	Total cholesterol taken in food.	Duration of diet period.	Total weight of un-saponifiable matter.	Total weight of cholesterol and copro-sterol passed in fæces.	Deficit.	Output per cat per day.
	grammes.		grammes.	grammes.	grammes.	
I	2·7	14	2·554	1·397	1·303	0·098
II	2·56	14	3·02	1·077	1·483	0·077
III	1·35	14	1·331	0·5486	0·8	0·032
IV	3·5	28	4·3985	1·4004	2·099	0·05

It is clear from these results that cats behave similarly to dogs when fed on meat diets, but the tendency for the change of cholesterol into coprosterol appears to be greater in the case of cats.

The total cholesterol of the food should of course be increased by that poured into the gut in the bile during digestion. No data are, however, available for forming any estimate of these quantities.

In the hope of ascertaining whether the whole or any of the cholesterol of the bile was excreted in the fæces in the case of artifical diets as free as possible from cholesterol or phytosterol, and if so, whether under such conditions any reduction to coprosterol took place, the experiments detailed below were undertaken. We had some difficulty in finding suitable food, as cats are dainty animals and will not eat freely of substances that are in the least distasteful to them, and we thought that any attempt to starve an animal into eating any particular food would be likely to vitiate the results. Further, it was necessary that the diet should contain all the constituents required to keep the animal in good condition.

Ultimately the following diets were selected:—

(1) 90 grammes of white bread mixed with the white of one egg were moistened with a dilute solution of Liebig's extract of meat and lightly fried. About 4 grammes of cream were then added.

(2) About 200 grammes of germ of wheat, which had previously been thoroughly extracted with ether, were mixed with a little Liebig's extract dissolved in hot water to a stiff paste. This was incorporated with about 30 grammes of suet, and the paste baked for two or three hours in a dish in a hot oven. The suet used was purified as far as possible from cholesterol by dissolving in ether and precipitating with alcohol several times. An analysis showed that this purified fat still contained 0·118 per cent. of cholesterol either free or in the form of esters.

The animals experimented on partook of these foods readily and appeared to thrive on them.

V. A cat weighing 3·5 kilogrammes was fed for 17 days on the above-mentioned bread and egg diet, and the fæces were collected during the last 15 days. During the period in which fæces were collected, the animal ate 1390 grammes of bread, the whites of 18 eggs, and about 65 grammes of cream. The weight of the cat remained quite constant until the 10th day of the experiment, after which it gradually decreased to 3·2 kilogrammes; 300 grammes of dry fæces were collected, which after extraction yielded 1·735 grammes of unsaponifiable matter as a dark oil. On recrystallisation from alcohol three times, 0·6075 gramme of white crystalline matter, melting at 125°—138° C. was obtained. From the residues 0·2604 gramme of benzoate was prepared. A microscopic examination of the crystalline matter showed that it was a mixture of cholesterol with probably some phytosterol-like substance from the bread. Reckoning the whole as cholesterol, the total weight obtained was 0·8126 gramme, corresponding to an output of 0·05 gramme per day.

VI. This cat was fed for 17 days on the bread-egg-cream diet, with the addition during part of the time of 2 grammes of cholesterol, given in 0·25-gramme portions. Altogether the animal ate 1710 grammes of bread, the white of 17 eggs, and about 60 grammes of cream. It liked the food, and during the experiment increased in weight from 3·2 to 3·3 kilogrammes. The total weight of dry fæces was 488 grammes and yielded 2·48 grammes of unsaponifiable matter of a crystalline nature. After twice crystallising from alcohol, 1·935 grammes of white crystalline matter were obtained. This was again recrystallised from alcohol, and the main crop consisted of almost pure cholesterol, melting at 143°—144° C. The final mother liquors yielded a minute amount of matter, crystallising in star-shaped aggregates of needles, not unlike coprosterol in appearance. The residues, on benzoylation in pyridine, yielded 0·1723 gramme of benzoates. Assuming that the whole crystalline matter consisted of cholesterol, the total amount was 2·07 grammes, a quantity only a little greater than the weight of pure cholesterol given to the animal.

VII. Four cats were fed for 10 days on the above-mentioned diet of extracted germ of wheat and purified fat, the fæces being collected during the last nine days. The animals took the food readily and ate during the period 1980 grammes of wheat germ and 308 grammes of fat, the total weight of which, when cooked as described, was 3916 grammes. The weights of the cats during the experiments were as follows.

490 grammes of dry fæces were collected and yielded on extraction 3·3246 grammes of unsaponifiable matter of an oily semi-solid consistency.

After several crystallisations from alcohol, 1·1495 grammes of white

	First day.		Third day.		Sixth day.		Ninth day.	
	lbs.	ozs.	lbs.	ozs.	lbs.	ozs.	lbs.	ozs.
Cat I	7	14	7	14	8	0	8	2
„ II	7	6	7	6	7	4	7	2
„ III	6	14	6	10	6	4	6	2
„ IV	5	14	5	12	5	6	5	4

crystalline matter were obtained, which melted at 135°—137° C. This consisted mainly of cholesterol, for a portion, after recrystallisation again from alcohol, melted at 142° C., and another portion, on treatment in ether acetic acid solution with bromine, according to Windaus' method, gave cholesterol dibromide, melting at 120°—122° C., in fair yield. The mother liquors, after recrystallisation from alcohol, yielded a small amount of matter, which under the microscope had the appearance of a mixture of cholesterol and phytosterol. The residues, after separating the above-mentioned 1·1495 grammes of cholesterol, were benzoylated in pyridine solution and 0·5049 gramme of fairly clean benzoate was obtained. This melted, after recrystallisation from ethyl acetate at 146°—147° C., to a turbid liquid, which cleared at 170° and on cooling gave a brilliant display of colours. Reckoning the whole of the crystalline matter as cholesterol, 1·5472 grammes were obtained, corresponding to a daily output of about 0·04 gramme; or, if we subtract the quantity of cholesterol contained in the fat given with the wheat germ, which amounted in all to 0·36 gramme, the daily output, independent of food, was 0·033 gramme. No trace of coprosterol was discovered.

VIII. A cat, weighing 1·7 kilogrammes, was fed on a diet prepared similarly to the last, but without any fat, for 17 days. It consumed altogether 630 grammes of extracted germ of wheat, and produced 93 grammes of dried fæces. The weight of the unsaponifiable matter was 0·6930 gramme and fairly crystalline. From this 0·5495 gramme of cholesterol was obtained, corresponding to an output per day of about 0·03 gramme.

IX. A cat was fed as in Experiment VIII for eight days, but during the first five days it received, mixed with its food, small quantities of pure phytosterol. It consumed altogether 250 grammes of the extracted germ and 1·41 grammes of phytosterol. The weight of dry fæces was 68 grammes and this yielded 1·7415 grammes of unsaponifiable matter as a greasy crystalline solid. On crystallisation from alcohol, 1·3545 grammes of white crystalline matter, which appeared to consist of almost pure phytosterol. An attempt was made to separate any cholesterol from this by conversion into the dibromides by Windaus' method, but without success. A small quantity of dibromide separated out on standing after the addition of the acetic acid solution of bromine to the solution of the substance in ether; this was filtered off and reduced in glacial acetic acid solution with zinc dust. The product, which should have been cholesterol, had it been present in any quantity, on heating began to soften at 137° and was not completely melted until 142° C. An examination of the crystals under the microscope showed that they contained phytosterol. The soluble dibromide treated in a similar way gave a substance melting at 138°—140° C. A microscopic examination showed that this was largely phytosterol.

The residues, after the separation of the 1·3545 grammes of phytosterol, were treated in pyridine solution with benzoyl chloride; 0·1196 gramme of benzoate was obtained. This, after recrystallisation from alcohol, melted at 145°—146° C. to a *clear* liquid and on cooling showed colours, though not very brilliantly. On carefully examining the crystals under the microscope, they were found to consist of phytosterol benzoate and none of the typical square plates of cholesterol benzoate could be seen. The conversion of phytosterol into the benzoate by the pyridine method is by no means quantitative, so that we do not know whether all was recovered from the residues. Altogether 1·4487 grammes of phytosterol were recovered, including, of course, cholesterol if present. All the phytosterol given in the food was, therefore, excreted unchanged, but whether accompanied by cholesterol we cannot say. There could not, however, have been much.

X. Immediately after the conclusion of the last experiment the diet was continued, but with the substitution of cholesterol for phytosterol. The experiment lasted 12 days, cholesterol being given with the food on the first eight days only. During the period the animal ate 620 grammes of germ and 2 grammes of cholesterol; 138 grammes of dried fæces were collected and the yield of unsaponifiable matter was 2·1775 grammes in the form of a brown solid. After recrystallising twice from alcohol, 1·5455 grammes of cholesterol, melting at 145°—147° C., were obtained. This figure is rather low owing to an accident, but the amount lost was under one-tenth of a gramme. The residues on benzoylation yielded 0·4386 gramme of cholesterol benzoate. The total cholesterol obtained was, therefore, 1·89 grammes, so that the total amount excreted could not have been greater than the weight of the cholesterol administered.

Discussion of the Results.

The conversion of cholesterol into coprosterol in the gut of the cat appears to take place only in the case of meat diets, and then the change is not necessarily complete. The two cats in Experiments I and II which yielded only coprosterol had been previously fed for some time on sheep's brain. The others, which had previously been fed in an ordinary way and led the ordinary domestic life, yielded a mixture of cholesterol and coprosterol. The animals fed on the artificial and vegetable diets gave no coprosterol. This recalls the experiences of Müller,* who found that in man a prolonged milk diet resulted in the excretion of cholesterol and not coprosterol. In all our experiments on meat diets the total cholesterol and coprosterol excreted was considerably less than that taken in with the food. Without considering the cholesterol poured into the gut with the bile, the percentage loss in Experiments I and II together was 53 per cent.; in Experiment III, 40 per cent.; and in Experiment IV, between 59 and 60 per cent., an average loss of 0·08 gramme per day. Two alternative explanations of these results suggest themselves.

(1) The hypothesis put forward in an earlier paper† that cholesterol is a substance which is strictly conserved in the animal economy: that when the destruction of the red blood corpuscles and possibly other cells takes place in the liver, their cholesterol is excreted in the bile, and that the cholesterol of the bile is reabsorbed in the intestine along with the bile salts, and finds its way into the blood stream to be used in cell-anabolism; and further, that any waste of cholesterol might be made up from that taken in with the food. This latter process would of course be limited in man and carnivorous animals by the change of cholesterol into coprosterol, and in herbivorous animals by the fact that their normal food does not contain cholesterol, but isomeric substances such as phytosterol, which would have to be converted into cholesterol before utilisation. Further evidence in support of this hypothesis in the case of herbivorous animals was brought forward by Miss Fraser and one of us in a paper‡ on the "Inhibitory Action of the Sera of Rabbits fed on Diets containing varying Amounts of Cholesterol on the Hæmolysis of Blood by Saponin."

(2) The change of cholesterol into coprosterol is generally supposed to be one of simple reduction brought about in the intestine by the bacteria of putrefaction. We have, however, no experimental evidence that coprosterol is a simple reduction product of cholesterol, as it is quite different from any of the bihydrocholesterol derivatives hitherto produced in the laboratory; and further, attempts to bring about the change *in vitro* by means of bacteria have so far been unsuccessful. Whatever the exact nature of the change

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* "Reduction of Cholesterol to Coprosterol in the Human Intestine," 'Zeit. physiol. Chem.,' 1900, vol. 29, pp. 129—135.

† "Origin and Destiny of Cholesterol," Part III, 'Roy. Soc. Proc.,' B, vol. 81, 1909, p. 109.

‡ "Origin and Destiny of Cholesterol," Part V, 'Roy. Soc. Proc.,' B, vol. 81, 1909, pp. 230—247.

may be, however, it may be accompanied either by a total destruction of a portion of the cholesterol, which in view of the great chemical stability of the molecule of this substance is unlikely, or a change of a portion into some non-crystalline oily product.

We do not think, however, that a comparison of the total weights of the unsaponifiable matter of the fæces given in Table I with the weights of cholesterol in the food bears out the second explanation, more especially when we remember that the latter weights should be increased by the quantities of cholesterol poured into the gut with the bile during digestion. The weights of unsaponifiable matter are, moreover, generally higher than the truth, as they are often rather difficult to dry, without drastic means, and often contain traces of soap.

If the first-mentioned explanation were strictly true, we should not have expected to find any cholesterol in the fæces of the cats fed on the artificial cholesterol-free diets—the fæces should have been cholesterol-free, just as are those of herbivorous animals. Small quantities of cholesterol were, however, found. In Experiment V, on bread, egg, and cream diet, the cat excreted 0·05 gramme per day, a minute fraction of which, however, may have been due to cream; and further, in this weight is included the phytosterol of the bread. In the case of the cats on extracted germ of wheat, in Experiments VII and VIII, the quantities excreted were 0·033 and 0·03 gramme per day respectively. These values may also have contained traces of phytosterol left in the germ after extraction. Whether these quantities are large enough to represent the whole of the cholesterol of the bile daily poured into the intestine, no data are available to determine. If, however, we adopt the data given for dogs, the values are undoubtedly too low. Further, the quantities of fæces produced per day on the vegetable diets were very much larger for a given weight of food than in the case of meat diets, and possibly this may have caused some of the cholesterol to escape absorption.

In the case of Experiments VI, IX, and X, however, in which known quantities of cholesterol or phytosterol were added to the daily rations of the artificial foods, no excess of cholesterol above that administered was recovered from the fæces.

From the point of view of deciding whether in the case of carnivorous animals the cholesterol of the bile is normally reabsorbed along with the bile salts in the intestine, these results are inconclusive. Experiments are, however, in progress to compare the effect on the blood of the addition of cholesterol to artifical diets such as those used in the experiments detailed in this paper. The results of these experiments we expect to give more definite information on this point, and we hope to make the subject of a communication in the near future.

The expenses in connection with this work were defrayed by means of a grant made by the Government Grant Committee of the Royal Society, for which we take the opportunity of expressing our thanks.

On the Supposed Presence of Carbon Monoxide in Normal Blood and in the Blood of Animals anæsthetised with Chloroform.

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(From the Physiological Laboratory of the University of London.)

While engaged in the study of the gases of the blood during the various stages of anæsthesia by chloroform, we found after absorption of the carbon dioxide and oxygen extracted by the blood pump an amount of residual gas far in excess of any amount that could be regarded as nitrogen remainder plus leak of apparatus.* We have found as the result of many experiments, carried out to determine this particular point, that practically all the chloroform present in the blood of anæsthetised animals comes off with the gases of the blood when these are extracted at 40° C., so that the excessive residual gas is in large part chloroform vapour, or its decomposition products. The exact method of procedure of analysis of these gases and the effects of the presence of this chloroform on the methods of analysis will form the subject of a forthcoming paper, but we quote the following experiments to show what percentages of chloroform may be present:

Cat, weight 3 kilos.; chloroformed for one hour with an air-chloroform mixture 2—3 per cent., 54 c.c. of dark blood withdrawn from carotid artery.

The gases extracted with the pump at 40° C. were mixed with excess of pure moist oxygen and passed through red-hot spiral platinum tubes. The products of combustion were collected in ammonia. This was exactly neutralised with nitric acid and titrated with silver nitrate (1 c.c. = 0·001 Cl); 17 c.c. of silver nitrate were required = 0·01986 gramme $CHCl_3$ = 3·7 c.c. of chloroform vapour at 0° and 760 mm.

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* The blood pump employed was the Tœpler as modified by Barcroft ('Journal of Physiology,' vol. 25, p. 265), with certain modifications for this particular work. These will be described in our paper on the blood gases in chloroform anæsthesia.

The blood therefore contained 0·0348 gramme of chloroform per 100 grammes of blood, so that the blood gases evolved contain about 10 per cent. by volume of chloroform vapour.

To quote another experiment:—

Cat, weight 3·5 kilos.; chloroformed with an air-chloroform mixture of 2 per cent., 54 c.c. of dark blood withdrawn from carotid artery for analysis of gases. Immediately before and after withdrawing this, two extra samples were taken of 10 c.c. each for analysis by the method which we call the method of Nicloux.*

Volume of gases extracted = 30·53 at 0° and 760 mm. This gas was analysed as in former experiment; 19·9 c.c. of silver nitrate were required (1 c.c. = 0·001 Cl), which corresponds to 0·0223 gramme of chloroform = 4·15 c.c. of chloroform vapour at 0° and 760 mm. = 0·0419 gramme of $CHCl_3$ in 100 grammes of blood.

Sample I (Nicloux method) gave 0·0396 gramme of chloroform per 100 grammes of blood.

Sample II (Nicloux method) gave 0·0403 gramme of chloroform per 100 grammes of blood.

The blood gases were always evacuated from blood at 40° C., and at this temperature and under the conditions we employed for anæsthetisation it is clear that practically all the chloroform which is calculated to be in blood can be recovered as chloroform vapour.

In 1894 Gréhant† recognised the presence of a combustible gas in the blood. This he considered to be carbon monoxide. The amount of this in blood he determined with his grisoumètre, an instrument by means of which he could measure the quantity of carbon monoxide fixed by hæmoglobin in an atmosphere which contained one part in 60,000.

As the result of experiments carried out on dogs in Paris, Desgrez and Nicloux‡ stated that carbon monoxide is not only a normal constituent of the blood gases, but that the blood of these animals, when anæsthetised by chloroform, contained an augmented quantity of this gas: "Les animaux soumis à l'anesthésie par le chloroforme nous ayant fourni un sang notablement plus riche en oxyde de carbone que leur sang normal." This conclusion is drawn from their experiments, which we summarise in the following table.

Their method of estimating carbon monoxide consisted in passing the

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* "Dosage de Petites Quantités de Chloroforme," 'Extraits du Bulletin de la Société Chimique de Paris,' 3rd series, vol. 33, p. 321, 1906.

† Gréhant, 'Comptes rendus,' November 8, 1897, and 'Les Gaz du Sang,' p. 109, 1894.

‡ 'Archives de Physiologie,' No. 2, April, 1898, p. 377.

Table I.

	Weight of dog.	Volume of blood.	Normal. CO found.	Anæsthetic.	Intermittent anæsthesia.	Persistent anæsthesia.	CO found.	Normal. CO per litre.	Anæsthesia. CO per litre.	Remarks.
	kilos.	c.c.	c.c.		h. m.		c c.	c.c.	c.c	
Exp. I...	7·5	25	0·04	Alcohol-chloroform, 3 : 1	2 50 Interval of ½ hour.	—	0·073	1·6	1·9	
					h. m. 1 30 Interval of ½ hour.		0·063	—	2·5	
					h. m. 1 30		0·06	—	2·4	
Exp. II...	16	25	0·04	$CHCl_3$	—	h. m. 1 20	0·1	1·6	4	
						1 5	0·133		5·3	
						2 0	0·16		6·4	
						1 0	0·173		6·9	
							0·093		3·7	3 hours after cessation of anæsthesia.
Exp. III...	10	25	0·036	$CHCl_3$	—	2 16	0·1	1·45	4	
						2 0	0·12		4·8	
							0·073		2·9	2 hours after cessation of anæsthesia.

blood gases over iodine pentoxide at 150° C., and estimating the iodine liberated by the method of Rabourdin. Beyond the fact that carbon monoxide decomposes iodic anhydride under these conditions, whereas neither hydrogen nor methane will do this, Desgrez and Nicloux bring forward no evidence whatever that the liberation of iodine in their experiments is due to carbon monoxide from the blood.

They explain the production of carbon monoxide in chloroform anæsthesia, in the light of observations of Desgrez,[*] that chloroform is decomposed by a solution of potash 1 : 8 with production of carbon monoxide according to one of the following equations:—

$$CHCl_3+2KOH = 2KCl+H_2O+HCl+CO;$$
$$CHCl_3+KOH = KCl+2HCl+CO.$$

But the blood is certainly not an alkaline fluid in the sense that even an extremely dilute solution of potash is: the blood is both alkaline and acid according to the indicator chosen. This reaction cannot therefore be regarded as lending support to their conclusions.

The statement that carbon monoxide is contained in blood was apparently confirmed by Saint-Martin.[†] This observer worked with large quantities of blood, 500 c.c. being taken for an experiment. The blood was mixed with oxalate of potassium solution in the proportion of 1 gramme of the salt to 500 grammes of blood. The gases evacuated from this mixture at 45° C. by the blood pump were rejected, and on the subsequent addition of 250 c.c. of saturated tartaric acid solution a further liberation of 30 to 40 c.c. of gas occurred. This proved to be a complex mixture of gases among which was carbon monoxide, which he absorbed with ammoniacal cuprous chloride.

In two papers, Lepine and Boulud[‡] state that in 25 c.c. of dog's blood they could find no carbon monoxide, though working with the apparatus devised by Desgrez and Nicloux. In blood taken *post mortem* of severe cases of anæmia, carbon monoxide is present, whereas in control cases which were non-anæmic the presence of this gas could not be detected. After injections of oxalate of calcium or tartaric acid into the circulation as much as 0·4 c.c. of carbon monoxide per 100 c.c. of blood was found. Practically no experimental details however are given in their papers.

Finding all these results difficult to understand, we incidentally, during the course of some other experiments, made an examination of a sample of the blood of a cat which had undergone a prolonged anæsthesia, both spectroscopically and by Haldane's method, without being able to detect the presence of any carbon monoxide; and without denying the conclusions of Desgrez and Nicloux, stated in a paper published in 1906,[*] that "we were unable to accept the view that the combustible gas appearing during chloroform-narcosis is carbon monoxide, a product of the decomposition of chloroform within the organism." This led Prof. Nicloux, in his work 'Les Anesthesiques Generaux,'[†] to make an attack on us and represent that we had denied the accuracy of his results on the strength of a single experiment by a spectroscopic method on a cat, whereas his experiments, he remarks, were performed on dogs. In reality our observations were made by the admittedly delicate method of Haldane, in which weak solutions of blood are examined in long glass tubes. We were therefore led to examine the conclusions of Desgrez and Nicloux in more detail.

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[*] 'Comptes Rendus,' November 15, 1897.

[†] 'Comptes Rendus,' February 14, 1898.

[‡] Lepine et Boulud, 'Comptes Rendus,' p. 56, 1905, and p. 302, 1906.

Is there any carbon monoxide in the gases of the blood which can be detected by passing the gases through solutions of oxy-hæmoglobin?

It will be seen from the table we have given that Desgrez and Nicloux state that normal blood contains 1·6 c.c. of carbon monoxide per litre of blood. In that of anæsthetised animals they find amounts of 2·6 c.c., 2·4 c.c., and 6·9 c.c. On the assumption that a litre of blood yields 600 c.c. of mixed gases at 0° and 760 mm., then the CO-content of normal blood is 0·27 per cent., which in chloroform anæsthesia may reach 1·15 per cent. by volume. The actual quantities of gases these observers examined were about 15 c.c.

We have minutely followed the directions contained in Haldane's papers,[‡] using mixtures of air and carbon monoxide. In the first experiments 500 c.c. of air with 4 c.c. of carbon monoxide = 0·8 per cent. CO were used. Measured volumes of this mixture were slowly bubbled through 1 : 100 solutions of freshly defibrinated cat's blood. The tubes so treated were compared with control solutions, the two solutions being compared either undiluted or equally diluted. Long columns of these solutions were examined in long glass tubes, with the following results:—

Series.	Volume of mixture of air + CO in c.c.	
I	50	Hb + CO detected without dilution.
	25	Ditto.
	15	Ditto.
II	50	Ditto.
	25	Ditto.
	10	Ditto.
	5	Detected on dilution in long tubes.

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[*] 'Roy. Soc. Proc.,' B, vol. 78, 1906, p. 414.

[†] Paris, 1908.

[‡] 'Journal of Physiology,' 1898, vol. 18, and *ibidem*, 1899, vol. 19.

In another series of experiments mixtures of 500 c.c. of air and 1 c.c. of carbon monoxide = 0·2 per cent. by volume were used.

Volume of mixture employed in c.c.	
50	Hb + CO recognised readily: 0·4 c.c. of the blood in water compared with control similarly prepared.
25	Equally well marked: 0·4 c.c. of blood in water compared with control similarly prepared.
10	Difference from control quite noticeable.

From these results it is clear that the quantities of carbon monoxide stated by the French observers to be present in blood gases should be readily recognisable by this method, for *half the amounts* stated to be present in normal blood are recognisable.

In order to ascertain whether carbon monoxide was present in the blood of anæsthetised animals the gases of cat's blood, never less and generally more than 60 c.c. in volume, were evacuated from 108 c.c. or more of blood at 40° C., without the addition of any acid, until no further trace of gas could be obtained. The chloroform used for anæsthetisation was chloroform puriss. B.P. (made from acetone) washed with water, shaken with excess of anhydrous potassium carbonate, filtered and then distilled.

To quote some of the experiments:—

	Duration of anæsthesia.	Volume of very dark blood.
	h. m. h. m.	c.c.
I.	12 52—2 19	54
	2 19—2 47	54

The total gases were slowly bubbled through 1 : 100 fresh defibrinated cat's blood and this examined against control solutions both diluted and undiluted. The solutions were also examined with Michael's tintometer. No differences whatever could be detected either by ourselves or by four separate independent workers in the laboratory, all of whom were unacquainted with the purpose for which the observations were being made.

II. 191 c.c. of blood, from two cats deeply anæsthetised for 1 hour 6 mins. and 1 hour 40 mins. respectively, were evacuated. The gas so obtained was slowly bubbled through 1 : 100 blood solution and compared with control blood solution through which an artificial blood gas containing chloroform vapour was bubbled. No difference whatever in tint could be recognised with the naked eye either in diluted or undiluted solutions by ourselves or by five independent workers in the laboratory. This result was confirmed by comparisons made with Michael's tintometer. Differences could, however, be readily detected when 0·2 c.c. of blood of similar dilution 1 : 100 saturated with CO gas was added to either of the original solutions through which the blood gases or artificial mixture had been bubbled.

	Duration of anæsthesia. hrs.	Volume of blood. c.c.
III.	2	54
	20 min. later.	27

Gases bubbled through as before, examination as above. No difference whatever could be detected.

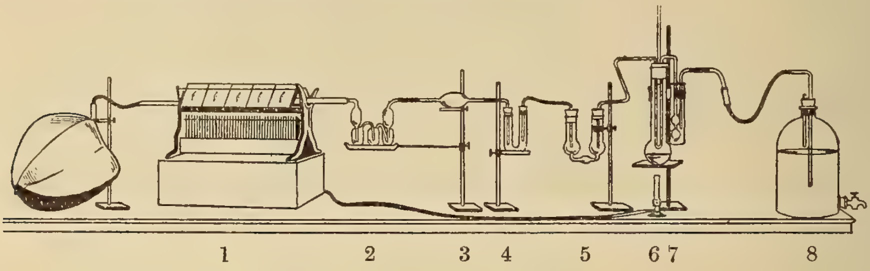
	Duration of anæsthesia. h. m.	Volume of blood. c.c.
IV.	1 30	54
	30 min. later.	54

The total gases as before bubbled through 1 : 100 human defibrinated blood. On examination as before, no differences whatever could be detected.

These experiments, we submit, conclusively show that when the blood of anæsthetised animals is evacuated at 40° C. until no further gas is evolved, the gas obtained contains no recognisable trace of carbon monoxide, and, therefore, if this is present it is present in far smaller quantities than Desgrez and Nicloux state to be the case in normal blood gases. Therefore, chloroform is not decomposed in the organism or in the blood with production of carbon monoxide. If there was any carbon monoxide really present in their experiments, this must have been due either to the acid which they added before evacuation of the gases, a view from which they explicitly dissent, or have been produced in their experiments.

It now became necessary to arrive at some explanation for the iodine evolved in the experiments of Desgrez and Nicloux, and the most obvious line of research was to study the effect of chloroform vapour on iodic anhydride at various temperatures, and also the effect of heat alone on this substance. For this purpose the following apparatus was employed. The gases of the blood, mixtures of air and chloroform or other gases under investigation were swept over the iodine pentoxide by a current of oxygen aspirated at a rate varying from 1 to 2 litres an hour. This plan was adopted to obviate the entrance of air of the laboratory, which might contain traces of carbon monoxide; and as a further precaution the oxygen was passed through a long combustion tube filled with red hot copper oxide.

The general arrangement of the apparatus is shown in the accompanying figure. The oxygen current passes from left to right through (1) combustion tube, (2) bulbs containing 40 per cent. potash, (3) bulb containing the gas under examination, (4) a U-tube containing powdered potash, the proximal limb of which was slightly moistened, (5) a calcium chloride drying tube, (6) tube containing iodic anhydride, (7) absorption vessel containing 10 c.c. of a 10 per cent. potassium iodide solution to absorb any liberated iodine (this was kept cool during the experiment), and (8) aspirator.



The bulb (3) was either one of Waller's densimeter bulbs of 255 c.c. capacity for mixtures of chloroform and air, or, in the case of blood gases, a 200 c.c. tube with stopcocks at either end.

Effect of Heat on Iodine Pentoxide.

Dr. Wade, of Guy's Hospital, who has for some time past been engaged in studying the methods of estimation of small quantities of carbon monoxide, kindly informed us, when we commenced these experiments, of the fact which he had observed that iodine pentoxide invariably gave off iodine when first heated, and that this evolution of iodine never actually ceased, although the rate eventually became steady if the temperature was maintained constant. This we can entirely confirm. We used iodine pentoxide tubes of a special kind devised by Dr. Wade, a full description of which, along with his results, he is about to publish. For this reason we give no further account of our experiments in this direction.

Effect of Chloroform Vapour on Iodine Pentoxide at various Temperatures.

The above-mentioned tubes were thoroughly cleaned and filled with 28 to 30 grammes of powdered iodine pentoxide and heated for a short time in aniline vapour, but in no case was any considerable quantity of water vapour evolved. The oxide was then heated in a current of purified oxygen for 24 to 48 hours or longer, the tube being placed in the vapour of a suitable liquid, boiling at the temperature we desired to use in an actual experiment, until the rate of evolution of iodine per hour became approximately constant. Different specimens of iodine pentoxide, and even different tubes made from the same sample, were found to differ considerably in this respect. These differences may in part have been due to traces of impurity in the iodine pentoxide, or more probably to different states of physical aggregation. A specimen obtained from a French source kindly recommended to us by Prof. Nicloux, which qualitative tests showed to be pure, after prolonged heating at 157° C. evolved iodine equivalent to 1 c.c. of N/1000 sodium thiosulphate in two hours, but we never reduced the amount below this value. We hoped at first to be able to determine for each tube the constant loss of iodine per gramme per hour at the temperature employed in our particular experiments, but experience soon showed that, in addition to the difficulty of keeping all conditions sufficiently constant, when chloroform vapour had been passed through a tube the "constant" underwent change. It was therefore found more satisfactory to make a blank determination of the iodine liberated in a given time by heat alone before each experiment and sometimes also after. The temperatures we employed were 100° C., the boiling point of xylene (137° C.), the boiling point of bromobenzene (157° C.).

The chloroform used in our experiments was made from acetone and carefully purified for us by Dr. Wade. It boiled at 61°·14 to 61°·15 C. at 760 mm. and had a specific gravity 1·5008 15/15.

Experiment I.—0·0588 gramme of chloroform, weighed in a thin bulb, was placed in the vessel (3) and the bulb broken. The chloroform vapour was swept over the iodic anhydride in a current of oxygen at the rate of 1 to 1½ litres per hour. The current was maintained for 81 minutes. Iodine equivalent to 102·9 c.c. N/1000 sodium thiosulphate was liberated. In a control experiment which had previously been made under similar conditions in 104 minutes, iodine was liberated equivalent to 1·5 c.c. N/1000 sodium thiosulphate. Thus each gramme-molecule of chloroform liberated 2·6 grammes of iodine, *i.e.* about 1/100 of a gramme-molecule of iodine.

In the following experiments, mixtures of air and chloroform vapour (amounting to 255 c.c. in each case), the concentration of which was determined by Waller's densimetric method, were passed through the apparatus at different temperatures. A few of the results are given in the following table (Table II).

A very large number of experiments were made, and it was found that the quantities of iodine liberated varied markedly with different tubes and with alteration of the conditions of the experiment. This was particularly the case in experiments conducted at 100° C.

Table II.

Temp.	$CHCl_3$ in air.	Duration of oxygen current in control.	Duration of oxygen current in experiment.	Iodine liberated in N/1000 sodium thiosulphate in		Iodine liberated by $CHCl_3$ only in terms of thiosulphate.	Remarks.
				Control.	Experiment.		
°	per cent.	h. m.	h. m.	c.c.	c.c.	c.c.	
157	7·9	1 35	2 5	3·4	57·45	51·55	Gas in aspirator smelt of $CHCl_3$.
137	4·0	2 0	1 20	4·5	8·5	4·0	In these experiments N/1000 was actually used, so that the figures are only approximate. The gas in aspirator smelt distinctly of chloroform.
137	7·0	1 20	1 20	3·0	4·0	1·0	
100	9·2	1 40	1 30	1·5	24·0	22·65	

[*October* 12, 1909.—"It was further noticed when the U-tube (4) containing solid potash was eliminated from the circuit, the quantities of iodine evolved underwent a marked diminution.

When iodine pentoxide is boiled under a reflux condenser with pure chloroform the action is very slow. One gramme of pentoxide boiled with 5 c.c. of pure chloroform for three hours only gave iodine equivalent to 18·7 c.c. of N/1000 sodium thiosulphate. At higher temperatures the action does not appear to proceed much more rapidly, as the following results of experiments in which iodine pentoxide and chloroform were heated in sealed tubes at temperatures of 100° C. and 137° C. respectively indicate:—

(1) Weight of I_2O_5.	Volume of chloroform.	Temperature.	Duration of heating.	Iodine liberated in terms of N/1000 thiosulphate.
gramme.	c.c.		hour.	c.c.
1	1	100	1	13·9
1	1	137	1	21·1

In control experiments in which the same weights of iodine pentoxide were heated without chloroform, the quantities of iodine set free were equivalent to 1·2 and 1·4 c.c. of thiosulphate respectively.

It was clear from these results that although iodine pentoxide reacts with pure chloroform with the liberation of iodine, the amounts so produced are insufficient to account for some of the quantities found in the experiments quoted in Table II.

It is known from the experiments of Desgrez*, and of Thiele and Dent†, that chloroform is decomposable by aqueous potash in the cold with the production of carbon monoxide, and it seemed not unlikely that in this reaction an explanation was to be found for the comparatively large amounts of iodine evolved when U-tube (4) was in the circuit.

That this was the case is proved by the following experiments:—

Effect of Solid Potash on Chloroform.

Experiment I.—Two stout test-tubes filled with mercury were inverted in a bath of mercury. Into one a piece of dry solid potash was introduced, and into the other a piece of potash, the surface of which was moistened with water. A few drops of chloroform were then passed into each tube. In both cases a slow evolution of gas commenced at once, and after standing overnight 3—4 c.c. of gas had collected above the mercury. This gradually increased in quantity until at the end of three days the tubes were almost full of carbon monoxide.

Experiment II.—In this experiment a comparison was made between the quantities of iodine liberated when a 5 to 6-per-cent. mixture of chloroform and air was passed over iodine pentoxide (A) with the U-tubes 4 and 5 in the apparatus figured above filled with calcium chloride, (B) with the U-tubes 4 and 5 filled with roughly-powdered potash, that in the first limb of 4 being slightly moistened. The experiments were conducted otherwise under rigidly similar conditions. The following results were obtained:—

Expt.	Temp.	Composition of $CHCl_3$ and air mixture.	Duration of oxygen current in control.	Duration of oxygen current in expt.	Iodine liberated in terms of N/1000 thiosulphate in		Difference between expt and control in c.c. of thiosulphate.
					Control.	Expt.	
	° C.		hours.	hours.	c.c.	c.c.	
A using $CaCl_2$ tubes	137	5·95	2	2	4·4	11·25	6·85
B using KOH tubes	137	5·41	2	2	4·4	185·5	181·5

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* 'Comptes rendus,' 1897, vol. 125, p. 782.

† Thiele and Dent, 'Annalen,' 1898, vol. 302, pp. 223—274.

Experiment III.—1·5 litres of a 2-per-cent. chloroform-air mixture were driven by means of a current of purified oxygen through a Y-tube, one limb of which was connected with a U-tube containing calcium chloride A, the other with a U-tube containing roughly-powdered potash B. After passing through these tubes the gas was allowed to bubble slowly through two small wash bottles, each containing 30 c.c. of a freshly prepared dilute solution of human blood. The apparatus was so arranged that the gas passed through the parallel U-tubes and thence through the wash bottles at the same rate. At the end of the experiment it was found that the blood through which the gas from tube A (calcium chloride) had passed contained *no trace* of carboxyhæmoglobin, but that in connection with tube B (potash) was almost wholly converted from oxyhæmoglobin to carboxyhæmoglobin."]

Effect of Blood Gases in Anæsthesia on Iodine Pentoxide.

From a large number of experiments we give a few results in the following table. In all cases there was a minimal amount of experimental interference with the animal. The blood was always taken from the carotid artery. The gases were evacuated at 40° C. Percentage of chloroform inhaled = 2·3 per cent. CO_2 absorbed before experiment, and U-tube 4 always in circuit :—

Table III.

Weight of cat.	Duration of anæsthesia.	Volume of blood taken.	Temp. of experiment.	Duration of oxygen current in		Iodine liberated in terms of N/1000 sodium thiosulphate in		Iodine liberated owing to $CHCl_3$.
				Control.	Experiment.	Control.	Experiment.	
kilos.	mins.	c.c.	°	min.	min.	c.c.	c.c.	c.c.
2·8	48	54	157	120	120	2·68	18·81	16·13*
2·8	55	54	157	112	222	1·8	41·45	37·88 †
3·5	78	54	100	120	120	1·5	9·0	7·5

* Blood very dark. † Blood very dark. Animal near asphyxia.

It seems clear, therefore, from all these experiments, that the iodine liberated by the blood gases of an animal anæsthetised by chloroform is due partly to the vapour of chloroform and partly to the carbon monoxide produced by the action of chloroform vapour on the potash in U-tube 4, but *not to the carbon monoxide that might be liberated in the organism.*

In order to obtain further confirmation of this, experiments were made in which the blood gases before analysis were freed from chloroform by shaking with a few cubic centimetres of alcohol in the laboratory tube of the gas analysis apparatus, and, finally, from traces of alcohol vapour by shaking with ice-water. We quote one experiment:—

Weight of cat.	Duration of anæsthesia.	Volume of blood.	Temp.	Duration of oxygen current in		Iodine liberated in terms of N/1000 sodium thiosulphate in	
				Control.	Experiment.	Control.	Experiment.
kilos. 3	hour. 1	c.c. 54	° 151	min. 135	min. 135	c.c. 1·7	c.c. 2

The gas, therefore, liberated iodine equivalent only to 0·3 c.c. N/1000 thiosulphate, a quantity which may very well be ascribed either to the incomplete elimination of the chloroform or to the variation of the control value of the tube.

[*October* 12, 1909.—"Finally, in order to settle the question quite conclusively, we made the following experiment:—

A cat weighing 3·6 kilos. was anæsthetised by means of carefully purified chloroform for 1 hour 15 minutes; 108 c.c. of blood were withdrawn in two lots of 54 c.c. from the carotid artery. The gases were completely pumped out of this blood at 40° C., and divided into two portions. The one half was slowly bubbled through 1:100 blood solution, and examined by Haldane's method in the manner described in the earlier part of this paper. No trace of carboxyhæmoglobin could be detected.

The other half of the blood gas was allowed to stand for 45 minutes in the laboratory tubes of the gas analysis apparatus in the presence of a few lumps of moistened potash. It was then transferred to the measuring tube, and thence bubbled through 1:100 blood solution as before. The presence of carboxyhæmoglobin was obvious in this without dilution.

The results were confirmed spectroscopically in both samples before and after the addition of reducing agents."]

Conclusions.

1. Our experiments lend no support to the view that carbon monoxide is a normal constituent of the blood gases. We think that the small quantities of iodine found in Desgrez and Nicloux' experiments are due to the decomposition of iodine pentoxide at the temperature (150° C.) of their experiments.

2. Chloroform is not decomposed in the blood with the formation of carbon monoxide.

3. [*October* 12, 1909.—" The iodine liberated in the experiments of Desgrez and Nicloux on anæsthetised animals was due to some extent to the direct decomposition of the iodine pentoxide by the chloroform vapour contained in their blood gases, but mainly to the carbon monoxide produced by the action of this chloroform on the potash over which they passed the blood gases in order to free them from carbon dioxide. This explanation is quite in accordance with their observations that the amount of iodine liberated increases with the duration of the anæsthesia."]

We express our thanks to the Government Grant Committee of the Royal Society for the funds which they have placed at our disposal for our work.

The Hexosephosphate formed by Yeast-juice from Hexose and Phosphate.

By W. J. Young (Biochemical Laboratory of the Lister Institute of Preventive Medicine).

(Communicated by A. Harden, F.R.S. Received July 30, 1909.)

It has been shown by Harden and Young* that—(1) the rate of fermentation of glucose by yeast-juice is greatly increased by the addition of a soluble phosphate; (2) this rate soon diminishes until a constant rate is attained, which is only slightly greater than that of the original yeast-juice and glucose; (3) during this period of increased fermentative activity, the phosphate undergoes some alteration, and at the end of the period is no longer present in a form precipitable by magnesium citrate mixture. The authors suggested in the first paper that a combination of the phosphate with the sugar, to form a phosphoric acid ester, had taken place, and more recently† embodied this suggestion in the form of an equation—

$$2C_6H_{12}O_6 + 2R'_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R'_2)_2 + 2H_2O.$$

It has also been shown that the same phenomena occur when fructose or mannose is used in place of glucose.‡

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* 'Chem. Soc. Proc.,' 1905, vol. 21, p. 189; 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 405.

† 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

‡ 'Chem. Soc. Proc.,' 1908, vol. 24, p. 115; 'Roy. Soc. Proc.,' B, vol. 81, 1909.

In a paper on the changes that take place in the phosphorus compounds of plants, etc.,* Iwanoff stated that, during the fermentation of sugar by pressed yeast, the inorganic phosphates of the yeast are converted into organic compounds. This observation, however, remained unknown to the author of the present paper until the publication of a later paper by Iwanoff,† in which he described the formation of a phosphoric acid compound during the fermentation of glucose or fructose in the presence of sodium phosphate by yeast which had been previously treated with acetone ("zymin"). By precipitating with copper acetate, decomposing with sulphuretted hydrogen and evaporating, he obtained the free acids, corresponding to these compounds, in the form of dark brown syrups, which when prepared from glucose contained 19·6 to 20 per cent., and from fructose 13·4 to 14 per cent., of phosphorus. No other analyses were given in the paper. These syrups gave the characteristic reactions of aldo- and keto-groups, and yielded osazones when heated with phenylhydrazine, that from the glucose compound melting at 142°, and that from the fructose compound at 125°. Iwanoff concluded that these bodies were compounds of phosphoric acid either with a triose or with methylglyoxal, and that they were different according to the sugar from which they were derived.

At the time when Iwanoff's paper appeared, a considerable amount of work had been done by the author on this reaction, and a preliminary note was published describing the isolation of the compound by means of its lead salt.‡ It was shown that this salt had the empirical formula $C_3H_5O_2(PO_4Pb)$, and when decomposed with sulphuretted hydrogen yielded an acid which was slightly dextro-rotatory, and which, when the solution was boiled, underwent hydrolysis with formation of phosphoric acid and a lævorotatory reducing substance.

The present paper embodies the results of further work on this substance, and also on the similar compounds obtained by fermenting fructose and mannose in place of glucose. No difference has been detected between the compounds obtained from these three sugars.

Preparation of the Lead Compound.—A solution of 0·6 molar di-sodium or potassium hydrogen phosphate (R'_2HPO_4) was added to a mixture of yeast-juice and excess of glucose at 25°, in such an amount that rapid fermentation was set up. The quantity of phosphate which may be used varies with different juices,§ and the necessary amount was determined in each case in a small sample by measuring the rate of evolution of carbon dioxide under similar conditions. When the rate of fermentation had fallen, more phosphate was added, and the additions continued so long as this acceleration could be produced. The mixture was then boiled, and filtered from the coagulate formed.

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* 'Travaux de la Société des Naturalistes de St. Pétersbourg,' vol. 34, 1905.

† 'Zeit. physiol. Chem.,' 1907, vol. 50, p. 281.

‡ Young, 'Chem. Soc. Proc.,' 1907, vol. 23, p. 65.

§ Harden and Young, 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

In a typical experiment, a mixture of 440 c.c. of yeast-juice and 55 grammes of glucose was employed, and 440 c.c. of the potassium phosphate solution (37 grammes, K_2HPO_4) were added 80 c.c. at a time. When fructose is used in place of glucose, much more phosphate may be added; thus in one case a mixture of 495 c.c. of yeast-juice and 200 grammes of fructose was employed, and 1000 c.c. of the phosphate solution (104·4 grammes K_2HPO_4) were added, 100 c.c. at a time.

The liquid, after boiling and filtering, was always found to contain a little free phosphate, and this was removed by adding a solution of magnesium nitrate, making alkaline with potash, stirring well, and allowing to stand for a few hours. The filtered liquid was then neutralised with acetic acid, lead hexosephosphate precipitated by the addition of lead acetate, and washed repeatedly with water by means of a centrifugal machine, until the washings no longer reduced Fehling's solution. It was found that this precipitate invariably contained traces of some nitrogenous material, and it was therefore suspended in water, decomposed with sulphuretted hydrogen, and the excess of this gas removed after filtering by passing a current of air through the solution. The liquid was then neutralised with potash and the lead salt reprecipitated with lead acetate, the process being repeated until the precipitate, after washing, was free from nitrogen. Usually two or three such precipitations were found sufficient. The salt was then filtered off and dried, first on a porous plate and then in a vacuum over sulphuric acid. It was thus obtained as a white, amorphous powder, free from nitrogen, which, on decomposing with sulphuretted hydrogen, yielded an acid solution containing no free phosphate precipitable by magnesium citrate mixture.

The preparation of yeast sold under the name of "zymin," which is obtained by treating pressed brewers' yeast with acetone, may be used in place of yeast-juice for the preparation of hexosephosphate. In one experiment 10 grammes of zymin, 10 grammes of glucose, and 100 c.c. of water were incubated at 25°, and 75 c.c. of a 0·3 molar solution of potassium phosphate added, 15 c.c. at a time. After rapid fermentation had ceased the mixture was filtered without boiling, and the lead compound obtained from the filtrate as before.

Lead compounds have been prepared from glucose, fructose, and mannose, and the analyses of several different preparations of these compounds are given in Table I. The mannose employed was obtained by the hydrolysis of ivory-nut, and was purified by being converted into the phenylhydrazone, which was recrystallised from water and decomposed with benzaldehyde.

Table I.

No.	Origin of compound.	Weight taken.	CO_2.	H_2O.	$Mg_2P_2O_7$.	$PbSO_4$.	Percentages.			
							C.	H.	P.	Pb.
1	Glucose ...	0·5261	0·1870	0·0777	—	—	9·69	1·65	—	—
		0·6269	—	—	0·1775	0·5080	—	—	7·88	55·34
2	,, ...	0·4745	0·1696	0·0732	—	—	9·75	1·73	—	—
		0·5993	—	—	0·1704	0·4774	—	—	7·92	54·40
3	Fructose ...	0·3040	—	—	0·0890	0·2484	—	—	8·15	55·81
4	,, ...	0·4503	—	—	0·1337	0·3649	—	—	8·27	55·34
5	Mannose ...	0·3614	—	—	0·1021	0·2931	—	—	7·87	55·39
	$C_6H_{10}O_4(PO_4Pb)_2$ requires						9·60	1·33	8·27	55·20

Hexosephosphoric acid was obtained from the lead salt by suspending it in water, decomposing with sulphuretted hydrogen, and removing the excess of this gas from the filtered liquid by means of a current of air. An acid liquid was thus obtained which could be titrated with standard alkali, phenolphthalein being used as indicator, in a similar manner to phosphoric acid. In Table II several examples are given in which the amount of hexosephosphoric acid was estimated both by titrating the solution with decinormal alkali, and by decomposing with nitric and sulphuric acids, precipitating with magnesium citrate mixture, and weighing as magnesium pyrophosphate. The numbers all refer to 10 c.c. of the solutions.

Table II.

Origin.	Titration, c.c. N/10.	$Mg_2P_2O_7$.	Hexosephosphoric acid.	
			From titration.	From $Mg_2P_2O_7$.
Glucose	19·8	0·1086	0·1683	0·1664
,, ...	63·4	0·3458	0·5390	0·5296
,, ...	99·0	0·5434	0·8415	0·8323
,, ...	9·6	0·0517	0·0816	0·0792
,, ...	29·2	0·1559	0·2482	0·2388
Fructose	38·7	0·2124	0·3290	0·3253
Mannose	32·6	0·1802	0·2771	0·2761

When the solution was evaporated on the water-bath a charred mass was left which contained free phosphoric acid and had a strong odour of caramel, whilst even on evaporation under reduced pressure, or at ordinary temperatures in a vacuum over sulphuric acid, decomposition occurred and free phosphoric acid was formed. The solution was found to give Mohlisch's α-naphthol reaction. It reduced Fehling's solution only after some hours in the cold, rapidly on boiling : this reduction may be due to the hydrolysis of the compound with formation of a reducing hexose. This is known to take place, as an alkaline solution of the sodium salt that had been left standing for two or three days at room temperature was found to contain free phosphate, and a lævorotatory substance which reduced Fehling's solution in the cold in a few minutes. The same change was brought about more rapidly when the solution was boiled.

The acid was found to have a less reducing power when boiled with Pavy's ammoniacal copper solution than corresponded to an equivalent quantity of glucose. Thus five preparations from glucose gave reductions of 0·754, 0·788, 0·767, 0·732, and 0·800, an equivalent quantity of glucose being taken as 1. No osazone could be obtained, as on heating with phenylhydrazine acetate decomposition took place, and phenylhydrazine phosphate was formed. Attempts were also made to prepare insoluble hydrazones or osazones with phenylhydrazine, methylphenylhydrazine, benzylphenylhydrazine, parabromophenylhydrazine, and nitrophenylhydrazine, but in no case was any success attained.

No differences could be detected between the acids obtained from glucose, fructose, or mannose. They all yielded the same products on hydrolysis, formed salts having the same properties, and had approximately the same dextro-rotatory power. Table III gives the rotations of a number of preparations of these acids.

Table III.

No.	Origin.	Grammes per 10 c.c.	Observed rotation in 4-dm. tube. +	Temp.	α_D. +
1	Glucose	0·079	0·097	20°	3°·07
2	,, ...	0·539	0·671	18	3·11
3	,, ...	0·246	0·364	19	3·70
4	,, ...	0·114	0·161	16	3·53
5	,, ...	0·088	0·131	13	3·72
6	,, ...	0·178	0·278	16·5	3·90
7	,, ...	0·148	0·179	16	3·02
8	,, ...	0·247	0·333	15	3·38
9	,, ...	0·529	0·695	16·5	3·28
10	,, ...	0·425	0·560	25	3·29
11	Fructose	0·247	0·315	16·5	3·19
12	,, ...	0·314	0·416	18	3·31
13	,, ...	0·143	0·181	17	3·15
14	Mannose	0·152	0·189	19	3·12
15	,, ...	0·555	0·926	17	4·17
16	Yeast-juice	0·053	0·074	13	3·50

All these acids were prepared from the lead salts with sulphuretted hydrogen, excepting No. 13 which was obtained from the barium salt by means of dilute sulphuric acid. No. 16 was obtained from yeast-juice to which neither sugar nor phosphate had been added, whilst zymin was employed for the preparation of Nos. 6 and 11.

It will be observed that the differences between the values for the acids derived from fructose and mannose are of the same order as the differences among the glucose preparations themselves. With so low a rotatory power a small experimental error or a trace of optically active impurity would cause an appreciable difference in the α_D, and the differences seen in the table are probably due to these causes. The rotations of the barium salts of these acids, which are slightly soluble in cold water, were also determined, and were found to be approximately equal. These are given in Table IV.

Table IV.

Origin.	Grammes per 10 c.c.	Rotation in 4 dm. +	α_D 17°·5. +
Glucose	0·0665	0·085	3°·20
Fructose	0·0692	0·088	3·18
Mannose	0·0656	0·082	3·13

Hydrolysis of the Acid.

(a) *Production of Phosphoric Acid and a Lævorotatory Reducing Substance.*—When the solutions of the acid were boiled, phosphoric acid was gradually set free, the reducing power to Pavy's solution increased, and the solution became lævorotatory. Table V shows the rate at which the first two of these changes took place when a solution of hexosephosphoric acid prepared from glucose was boiled in a flask fitted with a reflux condenser.

The phosphoric acid is expressed as grammes of magnesium pyrophosphate

Table V.

Time, in hours.	Free phosphate.	Reduction.
0	0	0·258
2	0·167	0·297
4	0·238	0·342
7	0·304	0·371
11	0·365	—
15	0·401	0·373
27	0·467	0·371

and the reduction as the number of grammes of glucose to which it corresponded. The original solution contained 0·7167 gramme of the acid in 200 c.c. (approximately 0·01 molar), corresponding when completely hydrolysed to 0·511 gramme of magnesium pyrophosphate, and equivalent to 0·414 gramme of glucose. Samples were taken out after the intervals stated, and the free phosphate estimated with magnesium citrate mixture and the reducing power with Pavy's solution.

The solution after about seven hours' boiling became very dark coloured, some of the reducing substance formed evidently being decomposed.

The acids obtained from fructose and mannose behaved in a similar manner on boiling, in every case a lævorotatory substance and phosphoric acid were formed. The following Table VI gives a number of examples of this change in rotation on the hydrolysis, at 100°, of the compounds prepared from all three sugars and, in one case, No. 6, of that obtained from yeast-juice to which nothing had been added.

Table VI.

No.	Origin.	Time of boiling, in hours.	Rotation in 4-dm. tube.	
			Before. +	After boiling. −
			°	°
1	Glucose	6	0·161	0·299
		11	,,	0·558
		14	,,	0·658
2	,, ...	10	0·634	0·756
3	,, ...	6	0·543	0·924
4	Fructose	10	0·416	1·514
5	Mannose	6	0·230	0·204
6	Yeast-juice	16	0·074	0·500

As glucose is converted by alkalis into a lævorotatory mixture of glucose, mannose, and fructose, it was thought that the treatment with alkali in the separation of the free phosphate, during the preparation of the lead salt, might have altered the hexosephosphate in such a manner as to account for the lævorotatory sugar being obtained on hydrolysis. Preparations of the lead salt were therefore made without removing the free phosphate, the solution being kept always slightly acid, but the method being otherwise the same as before. The hexosephosphoric acids obtained from these acid preparations were found to have approximately the same specific rotatory power as the others. The strength of the solution used was estimated by determining the total phosphorus, and making allowance for the small quantity of free phosphoric acid, which was precipitated in a separate quantity with magnesium citrate mixture. In Table III, already given, Nos. 5, 6, 12, and 14 refer to acids prepared in this way, and it will be seen that they cannot be distinguished from those which had undergone the alkaline treatment. On hydrolysis, also, the same lævorotatory solution was obtained as is shown in Table VII.

Table VII.

No.	Origin.	Time of boiling, in hours.	Rotation in 4-dm. tube.	
			Before. +	After boiling. −
			°	°
1	Glucose	11	0·056	0·363
2	"	8	0·131	0·370
3	"	6	0·637	1·184
4	Fructose	2	0·317	0·276
	"	4	"	0·574
	"	6	"	0·956
	"	8	"	1·212

(b) *Nature of the Reducing Substance.*—To determine the nature of this reducing substance, the mixture from the hydrolysis of a preparation from glucose was exactly neutralised to litmus with barium hydrate, and the precipitation of the barium salts of phosphoric and unchanged hexose-phosphoric acids completed by the addition of two volumes of absolute alcohol. After filtration, the solution was evaporated at 35° to 40° under reduced pressure to a thick syrup. This was found to reduce Fehling's solution in the cold, and to be lævorotatory. It gave the red coloration characteristic of fructose when heated with resorcinol and hydrochloric acid (Seliwanoff). When this syrup was treated at 0° with milk of lime, an insoluble calcium compound similar to calcium fructosate was formed. This was filtered off, washed with water, suspended in water, and decomposed with carbon dioxide, and the filtered liquid treated with animal charcoal and evaporated under reduced pressure.

The colourless syrup thus obtained reduced an alkaline solution of copper glycocolate after 12 hours in the cold, a reaction which, according to Pieraerts,* is only given by fructose. It readily formed an osazone when heated with phenylhydrazine in the usual manner, and this, after recrystallisation, first from alcohol and then from a mixture of pyridine and water, melted at the same temperature as glucosazone, viz., 206°, whilst a mixture of this compound with glucosazone melted at 206° to 207°. The analysis also corresponded to that of glucosazone—

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* 'Chem. Zentral.,' 1908, vol. 1, p. 1854.

0·1222 gramme gave 16·2 c.c. nitrogen at 16°·4 and 774·3 mm.
$N = 15{\cdot}76$ per cent.

Glucosazone, $C_{18}H_{22}O_4N_4$, requires $N = 15{\cdot}64$ per cent.

The fructose and mannose acids, when treated as above, yielded syrups having the same properties as that from the glucose acid; in both cases glucosazone was obtained from them.

All these syrups were found to induce rapid fermentation in a mixture of yeast-juice and glucose with such an excess of phosphate that fermentation was only proceeding at a very slow rate, and this induction has been shown to be brought about by the addition of fructose, but not of glucose or mannose.*

Finally, the ratio of the rotation to the reducing power was obtained and was found to be approximately the same as that found for pure fructose. The reducing power was determined by means of Pavy's ammoniacal copper solution and, as this was standardised, each time it was used, with a solution of pure glucose it has been found convenient to express the reduction per 100 c.c. of solution by the number of grammes of glucose which would reduce the same amount of copper, and to compare the rotation of the solution in a 4-dm. tube with this number. In Table VIII, this ratio, $\frac{\text{Rotation}}{\text{Reduction}}$, is given for the hexoses obtained by means of milk of lime from the products of hydrolysis of the acids derived from all three sugars, and it will be seen that it agrees in every case fairly well with that found for pure fructose.

Table VIII.

Sugar from—	−Rotation in 4 dm. tube.	Reduction as glucose per 100 c.c.	Ratio.
	°		
Glucose acid	1·806	0·512	3·52
Fructose acid (1)	0·962	0·284	3·38
" (2)	0·516	0·151	3·41
Mannose acid	1·376	0·441	3·12
Pure fructose (1)	0·607	0·161	3·77
" (2)	0·829	0·219	3·79

The solutions, after removal of the phosphate and hexosephosphate and before the treatment with milk of lime, always possessed a greater reducing power than a solution of fructose having the same optical activity. The ratio of the rotation to the reduction was always lower than that for pure fructose, and varied from 1·36 to 2·78. This might be accounted for by the presence of glucose or mannose, and experiments were made to ascertain if these sugars were present; in no case, however, could either be detected. The insoluble hydrazone of mannose was not obtained with phenylhydrazine, nor was a hydrazone formed when the syrup was treated with methylphenylhydrazine in the manner described by Neuberg* for the separation of glucose or mannose from fructose. They were also examined for glucose by the method by which Lobry de Bruyn and van Ekenstein† detected glucose in the mixtures obtained by the action of alkalis on fructose or mannose. This consisted in removing most of the fructose by precipitating as calcium fructosate or by extracting successively with alcohol, ethyl acetate, ether, and acetone, and identifying glucose in the residue either by converting into α-methyl glucoside by means of methyl alcoholic hydrochloric acid, or by oxidising with dilute nitric acid and precipitating the acid potassium salt of the saccharic acid formed. Neither of these compounds could be obtained from the syrups under examination.

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* Harden and Young, 'Chem. Soc. Proc.,' 1908, vol. 24, p. 115; 'Roy. Soc. Proc.,' B, 1909.

In order to ascertain whether these low ratios were due to the action of the phosphoric acid on the fructose after it was set free, a mixture of these compounds in the same relative proportions as would be contained in hexosephosphoric acid was boiled for some hours, and the phosphoric acid then removed by means of barium hydrate and alcohol in a similar manner to that described for the hydrolysed acid. The results of two experiments are given in Table IX.

Table IX.

Time, in hours.	−Rotation in 4 dm. tube.	Reduction per 100 c.c.	Ratio.
(1) 0	7·424	1·927	3·87
10	3·832	1·082	3·54
15	2·580	0·791	3·26
(2) 0	0·711	0·188	3·78
10	4·250	1·276	3·33

In both these experiments the solutions became very dark coloured and a considerable quantity of fructose had been destroyed. It will be observed that in both cases the ratio became less on boiling, but the lowest value obtained was much higher than the numbers found for the product from hexosephosphoric acid. It is possible that the fructose as it is being formed is much more susceptible to the action of phosphoric acid. On the other hand, these low ratios may be due to the presence of some other reducing substances having a less lævorotatory power than fructose.

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* 'Ber.,' 1902, vol. 35, p. 959.

† 'Rec. Trav. Chim.,' 1895, vol. 14, p. 156.

Salts of Hexosephosphoric Acid.

In addition to the lead salt already described, several other salts have been prepared. Attempts to obtain the potassium and sodium salts were made by adding the hydrates to the acid solution until neutral to phenolphthalein, and evaporating the solution under reduced pressure. A sticky mass was left which would not crystallise, and which decomposed on keeping.

Solutions of the alkali salts slowly decomposed on keeping, free phosphate being formed and the solution becoming lævorotatory. This decomposition causes a small error in the estimation of the free phosphate present in a mixture of this salt and hexosephosphate by means of magnesium citrate mixture. Many of the salts are more soluble in cold than in hot water; thus, when magnesium citrate is added to a solution of sodium hexosephosphate, no precipitate is formed until the mixture is heated; a white precipitate then comes down, which redissolves on cooling. The manganese, barium, and calcium salts behave in a similar manner.

Silver Hexosephosphate, $C_6H_{10}O_4(PO_4Ag_2)_2$.—This salt was obtained by neutralising the free acid with caustic soda, adding the calculated quantity of silver nitrate and then half the total volume of alcohol. The precipitate was filtered off, well washed with a mixture of water and alcohol, dehydrated with alcohol and ether, and dried over sulphuric acid in a vacuum, and it was thus obtained as a white amorphous powder. It was exceedingly unstable, and the whole preparation was carried out by red light. Even when dry the salt darkened on exposure to light, whilst when heated with water it was at once reduced to metallic silver.

The following analyses were carried out with different preparations of this salt:—

Table X.

Origin.	Weight taken.	AgCl.	$Mg_2P_2O_7$.	Percentages.	
				Ag.	P.
Glucose (1)	0·2340	0·1740	0·0644	55·94	7·67
„ (2)	0·3976	0·2989	0·1057	56·54	7·40
Fructose (1)	0·3293	0·2439	0·0893	55·75	7·55
„ (2)	0·3214	0·2378	0·0909	55·70	7·87
$C_6H_{10}O_4(PO_4Ag_2)_2$ requires..............................				56·24	8·08

Barium hexosephosphate, $C_6H_{10}O_4(PO_4Ba)_2$, was obtained by adding a solution of barium chloride to a solution of potassium or sodium hexosephosphate, and warming the mixture on the water bath. A granular white precipitate was thus formed, which was found to be more soluble in cold than in hot water. This was purified by dissolving in cold water and reprecipitating by warming the solution. It was then filtered off, washed with warm water, and dried over sulphuric acid in a vacuum. At 17°·5 100 c.c. of solution were found to contain 0·665, 0·692, and 0·656 gramme of the barium salts derived from glucose, fructose, and mannose respectively The following analyses were obtained for this salt:—

Table XI.

Origin.	Weight taken.	$BaSO_4$.	$Mg_2P_2O_7$.	Percentages.	
				Ba.	P.
Glucose (1)	0·2578	0·1994	0·0960	45·50	10·36
,, (2)	0·5298	0·4002	—	44·46	—
	0·4317	—	0·1538	—	9·92
Fructose (1)	0·4009	0·3081	0·1485	45·23	10·32
,, (2)	0·5221	0·3933	0·1902	44·34	10·14
Mannose	0·4186	0·3179	0·1538	44·70	10·22
$C_6H_{10}O_4(PO_4Ba)_2$ requires				44·92	10·16

Calcium Hexosephosphate.—A calcium salt of the acid derived from glucose was obtained by adding calcium chloride to a solution of the sodium salt, and completing the precipitation by adding alcohol. When dried over sulphuric acid in a vacuum it gave the following analysis:—

0·3488 gramme gave 0·1958 gramme CO_2, 0·0917 gramme H_2O,
0·0890 gramme CaO, and 0·1778 gramme $Mg_2P_2O_7$.

C = 16·81, H = 2·92, Ca = 18·24, P = 14·20 per cent.

$C_6H_{10}O_4(PO_4Ca)_2+H_2O$ requires C = 16·59, H = 2·76, Ca = 18·41,
P = 14·28 per cent.

Source of the Hexosephosphate.—In order to show that this compound was formed from the added phosphate, two quantities of 50 c.c. of yeast-juice were incubated at 25°, one with 30 c.c. of a 30-per-cent. solution of glucose and the other with 30 c.c. of a 0·3 molar solution of potassium phosphate containing 30 per cent. glucose. After two hours both were boiled and filtered, 30 c.c. of each filtrate were treated with the same quantity of magnesium nitrate and potash to remove any free phosphate and, after standing for a few hours, the solutions were filtered, neutralised with acetic acid and lead hexosephosphate precipitated with lead acetate, washed and dried over sulphuric acid in a vacuum. The mixture to which the phosphate had been added gave 5·03 grammes of lead salt, whilst the other only yielded 1·35 grammes. This experiment also shows that yeast-juice itself contains a small quantity of hexosephosphate, and experiments have already been quoted to show that this compound has similar properties to that formed when phosphate and sugar are added. The same hexosephosphate was obtained by means of zymin in place of yeast-juice as already mentioned, and as in this case the mixture was not boiled before the precipitation of the lead salt, it follows that the salts of this acid were present in the mixture as such, and were not formed from a more complex body by the boiling, which was always necessary when yeast-juice was employed. A hexosephosphate has also been detected in the extract obtained by boiling pressed brewers' yeast with water.

Molecular Weight of Hexosephosphoric Acid.—The analyses of the salts of this acid gave no information as to its molecular weight, since the same percentage composition would be obtained if the acid had the formula $C_3H_5O_2(PO_4H_2)$ and not $C_6H_{10}O_4(PO_4H_2)_2$. The first compound might be expected on hydrolysis to yield glyceraldehyde or dioxyacetone, and these might polymerise to form hexoses; so that the fact that fructose was formed when the acid was hydrolysed does not do away with the possibility of the smaller formula.

As no derivatives of the acid could be obtained, such as a hydrazone or an osazone, analysis of which would have settled the question, recourse was made to physical methods. The molecular weight of the acid was calculated from the difference between the freezing point of its solution and that of water, and was compared with that calculated by taking into consideration the extent to which it was dissociated in solution as determined by the rate at which it hydrolysed cane sugar. The freezing points of three solutions of different concentrations of the acid prepared from glucose was found by the ordinary method, the amount of acid in solution was estimated by titration and checked by the total phosphorus content, and the weight of water in the solution was obtained from the specific gravity. By way of comparison with a compound of known constitution and of a similar nature, corresponding determinations were carried out with a sample of synthetical glycerophosphoric acid, which was purified by means of its lead salt in exactly the same manner as the hexosephosphoric acid. The results are given in Table XII, Nos. 1, 2, and 3 being those obtained from the hexosephosphoric acid, and Nos. 4 and 5 from two concentrations of glycerophosphoric acid.

Table XII.

No.	Normality.	Grammes per 100 c.c.	Specific gravity.	Weight of water.	Depression.	Molecular weight.
1	1·75	15·13	1·075	92·37	° 1·747	177
2	0·99	8·42	1·042	95·78	0·932	178
3	0·55	4·68	1·022	97·52	0·535	169·4
4	1·65	14·21	1·063	92·09	2·153	135·5
5	0·97	8·38	1·037	95·32	1·300	127·7

The formula $C_6H_{10}O_4(PO_4H_2)_2$ corresponds to a molecular weight of 340, and the smaller formula to 170, whilst glycerophosphoric acid, $C_3H_7O_2(PO_4H_2)$, has a molecular weight of 172. Thus the value obtained, although low, was much greater than that found for glycerophosphoric acid, which only differs in composition from the smaller formula by two hydrogen atoms.

The cane-sugar hydrolysis was carried out at 25° with semi-normal acid, and the rate was compared with that found for semi-normal hydrochloric and glycerophosphoric acids under similar conditions.

Table XIII gives the figures found for the hexosephosphoric acid and Table XIV those for the glycerophosphoric acid. Allowance has been made in the tables for the rotation due to the acids themselves. Solutions of the hexosephosphoric and glycerophosphoric acids of the same normality were also kept by themselves under similar conditions for the same time, and no alteration took place in their rotations.

Table XIII.—Hexosephosphoric Acid.

Time, in minutes.	Rotation.	x.	$A-x$.	$K \times 10^4$ $\left(K = \frac{1}{t} \log \frac{A}{A-x}\right)$.
0	° +26·83	0	34·88	—
30	25·95	0·88	34·00	3·700
60	24·98	1·85	33·03	3·945
90	24·01	2·82	32·06	4·069
120	23·12	3·71	31·17	4·070
160	22·05	4·78	30·10	4·001
180	21·44	5·39	29·47	4·051
210	20·66	6·17	28·71	4·026
240	19·95	6·88	28·00	3·976
310	18·17	8·66	26·22	3·999
330	17·72	9·11	25·77	3·984
360	17·06	9·77	25·11	3·965
390	16·32	10·51	24·37	3·993
420	15·70	11·13	23·75	3·974
∞	−8·05	34·88		
			Mean………	3·981

Table XIV.—Glycerophosphoric Acid.

Time, in minutes.	Rotation.	x.	$A-x$.	$K \times 10^4$.
0	+26·36	0	34·41	—
31	25·51	0·85	33·56	3·503
60	24·79	1·57	32·84	3·380
90	24·06	2·30	32·11	3·338
120	23·30	3·06	31·35	3·370
150	22·64	3·72	30·69	3·312
182	21·86	4·50	29·91	3·344
210	21·23	5·13	29·28	3·339
240	20·54	5·82	28·59	3·353
270	19·89	6·47	27·94	3·350
300	19·26	7·10	27·31	3·345
330	18·64	7·72	26·69	3·343
360	18·01	8·36	26·05	3·358
∞	−8·05	34·41		
			Mean.........	3·353

Semi-normal hydrochloric acid under the same conditions gave the constant $K \times 10^4 = 22·466$.

It follows from these rates that the ratio of hydrogen ions in the solution of hexosephosphoric acid to those in the hydrochloric acid was 3·981 to 22·466 or 17·7 to 100, whilst in the case of glycerophosphoric acid the ratio was 3·353 to 22·466 = 14·9 to 100. As hydrochloric acid is almost completely dissociated, these numbers may be taken as a measure of the concentration of hydrogen ions in the solutions. Glycerophosphoric acid is dibasic and a solution which is semi-normal by titration will contain only half as many molecules as a semi-normal solution of hydrochloric acid, or 50 molecules for every 100 of hydrochloric acid. If the glycerophosphoric acid be assumed to dissociate into two ions, then out of every 50 molecules 14·9 are dissociated into $14·9 \times 2 = 29·8$ ions, and $50-14·9 = 35·1$ molecules remain undissociated. The solution will thus contain $29·8+35·1 = 64·9$ units, and the freezing point depression will correspond to a molecular weight of $\frac{50}{64·9} \times 172 = 132·5$; those actually observed gave 135·5 and 127·7. Similarly with the hexosephosphoric acid, if the larger formula obtain, the acid is tetrabasic and a semi-normal solution will only contain one-quarter as many molecules as a semi-normal solution of hydrochloric acid, or 25 molecules to every 100 of hydrochloric acid. If the dissociation take place into two ions, of these 25 molecules 17·7 are dissociated into $17·7 \times 2 = 35·4$ ions, $25-17·7 = 7·3$ molecules remain undissociated, and the total number of units will be $7·3+35·4 = 42·7$. The molecular weight of the acid from the freezing point of this solution will be $\frac{340 \times 25}{42·7} = 199$; the observed values were 177, 178, and 169·4.

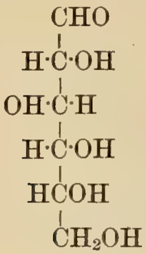
If, on the other hand, the compound have the smaller formula, with a molecular weight of 170, it will be a dibasic acid and the solution will contain 50 molecules to every 100 contained in a solution of hydrochloric acid of similar normality. The number of dissociated molecules corresponding to the hydrogen ions present will be 17·7, yielding $17{\cdot}7 \times 2 = 35{\cdot}4$ ions, $50 - 17{\cdot}7 = 32{\cdot}3$ will be undissociated, and the total number of units will be $35{\cdot}4 + 32{\cdot}3 = 67{\cdot}7$. The molecular weight, as deduced from the freezing point, would therefore be $\frac{170 \times 50}{67{\cdot}7} = 125{\cdot}5$.

The observed depression thus corresponds more closely to that which would be expected from the larger formula, although the agreement is not so good as that in the case of glycerophosphoric acid. This low result may be due to some hydrolysis in the solution, which would make the number of units still greater. If the hexosephosphoric acid had the smaller formula, it would be expected to depress the freezing point at least to the same extent as glycerophosphoric acid, since it only differs from this by two hydrogen atoms, and is slightly more dissociated in solution than this acid. These results thus point to the acid having the formula $C_6H_{10}O_4(PO_4H_2)_2$.

Further evidence against the acid being a derivative of glyceraldehyde is also afforded by the following facts:—When the acid was reduced with sodium amalgam, no glycerol was obtained, as might have been expected if it were a compound of glyceraldehyde. When glycerophosphoric acid was heated with hydriodic acid in a current of carbon dioxide, it was reduced to isopropyl iodide, which was detected by passing into alcoholic silver nitrate in the manner in which glycerol is estimated by the method of Zeisel and Fanto. On the other hand, hexosephosphoric acid gave no volatile iodide when treated in this manner.

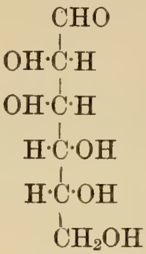
Constitution of Hexosephosphoric Acid.—It has not yet been found possible to obtain any evidence with regard to the position of the phosphoric acid groups in the molecule. The facts that the compound does not reduce Fehling's solution in the cold until after a long time, and that no hydrazones or osazones could be obtained, render it possible that the acid does not contain an active carbonyl group, and it may be that this group is involved in the reaction with the phosphoric acid.

Glucose, fructose, and mannose all appear to yield the same hexosephosphoric acid when fermented by yeast-juice in the presence of phosphate. One explanation of this is that these sugars are changed, before conversion into hexosephosphate, into some form common to all three. The only differences between glucose, fructose, and mannose are in the groups or arrangement of the groups attached to the first two carbon atoms:



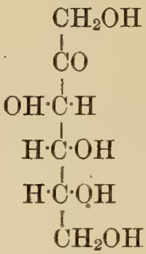
$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{H{\cdot}C{\cdot}OH} \\ | \\ \mathrm{OH{\cdot}C{\cdot}H} \\ | \\ \mathrm{H{\cdot}C{\cdot}OH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

Glucose.



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{OH{\cdot}C{\cdot}H} \\ | \\ \mathrm{OH{\cdot}C{\cdot}H} \\ | \\ \mathrm{H{\cdot}C{\cdot}OH} \\ | \\ \mathrm{H{\cdot}C{\cdot}OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

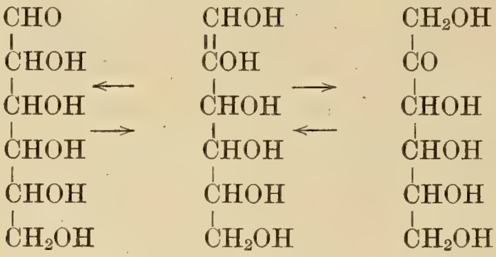
Mannose.



$$\begin{array}{c} \mathrm{CH_2OH} \\ | \\ \mathrm{CO} \\ | \\ \mathrm{OH{\cdot}C{\cdot}H} \\ | \\ \mathrm{H{\cdot}C{\cdot}OH} \\ | \\ \mathrm{H{\cdot}C{\cdot}OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

Fructose

and all these sugars have the same enolic form:



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_2OH} \end{array} \rightleftarrows \begin{array}{c} \mathrm{CHOH} \\ \| \\ \mathrm{COH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_2OH} \end{array} \rightleftarrows \begin{array}{c} \mathrm{CH_2OH} \\ | \\ \mathrm{CO} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

Glucose and mannose. Enolic form. Fructose.

and are converted into one another in alkaline solution,* and it is conceivable that the hexosephosphate is a derivative of this enolic form.

If the reaction take place according to the equation:

$$2C_6H_{12}O_6 + 2R'_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R'_2)_2 + 2H_2O,$$

two molecules of sugar are concerned, and another possibility is that the phosphoric acid groups are attached to a new molecule, which is formed by the combination of portions of each of these two sugar molecules. In this case, also, the hexosephosphoric acid might be expected to have the same composition when formed from different sugars.

Summary.—1. The compound formed during the accelerated fermentation of glucose, fructose, and mannose by yeast-juice, in the presence of a soluble phosphate, is a salt of an acid which probably has the formula $C_6H_{10}O_4(PO_4H_2)_2$, and may be isolated by precipitation of its lead salt.

2. The free acid may be obtained in solution by decomposing this lead salt with sulphuretted hydrogen.

3. The acid is very unstable and readily decomposes on keeping, or on evaporating even at ordinary temperatures in a vacuum over sulphuric acid, with formation of a reducing substance and phosphoric acid.

4. It reduces Fehling's solution only after some hours in the cold, rapidly on boiling, whilst no osazones or hydrazones have been obtained from it.

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* Lobry de Bruyn, 'Rec. Trav. Chim.,' 1895, vol. 14, p. 201.

5. No differences have been detected between the hexosephosphoric acids or their salts, whether derived from glucose, fructose, or mannose.

6. On hydrolysis of the acid by boiling, phosphoric acid is set free and fructose formed. No other hexose could be identified, but the solution, after hydrolysis, was always less lævorotatory than a solution of pure fructose of the same reducing power.

7. The salts of lead, barium, silver, and calcium have been prepared.

[The compound containing phosphorus, which was considered to be phenylhydrazine phosphate, has since been examined by von Lebedew ('Biochem. Zeits.,' 1909, vol. 20, p. 113), who regards it as a phenyl hydrazido-phosphoric acid compound of hexosazone. A re-examination of this substance by the author leads to the conclusion that it is in reality a derivative of hexosephosphoric acid, but decisive results as to its constitution have not yet been obtained.—*November* 15, 1909.]

The Comparative Power of Alcohol, Ether, and Chloroform as measured by their Action upon Isolated Muscle.

By Augustus D. Waller, M.D., F.R.S.

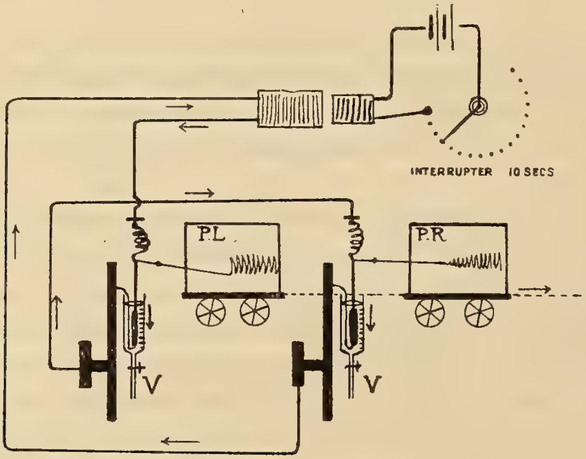
(Received and Read June 24, 1909.)

The object of the following communication is twofold: (1) to present the results of a careful comparison of the physiological effectiveness of certain narcotics, and (2) to illustrate the degree of accuracy of which such comparisons are susceptible by the systematic use of the sartorius muscle of the frog as an indicator.

Method.—The two sartorius muscles of a frog are dissected out and the portions of bone to which they are attached are ligatured with fine copper wires serving as conductors. The muscles are set up in the two vessels V, V and connected with two myographic levers that record their movements on two smoked plates L, R. The connections with the secondary coil of an inductorium (Berne model) are as given in the diagram, so that both muscles are traversed in series by the same current in the same direction. The muscles are directly excited once every 10 seconds by maximal break induction shocks. Each observation consists of three parts: a first part consisting of the normal responses of the muscle immersed in normal saline (0·6 per 100 NaCl in tap water); a second part consisting of the responses while the muscle is immersed in an experimental solution; a third part consisting of the responses while the muscle is replaced in normal saline. The solutions are changed by being run off through a tap and run in from a pipette, care being taken that the volume of fluid is always the same. The induction currents are kept going automatically throughout an experiment, excepting during the short periods required for changing the solution. The apparatus used for this purpose consists of: (1) a Berne coil fed by a 2-volt accumulator; (2) a Brodie clock with interruptions set at six per minute; and (3) a relay key, *i.e.* that shown by G. R. Mines at the July, 1908, meeting of the Physiological Society.

As a general rule of procedure in any comparison between the effects upon two muscles L and R of two solutions A and B, a first comparison is made between the effects of A on L and of B on R, and a second comparison of the effects of B on L and of A on R. Each complete experiment thus comprises two pairs of simultaneous trials of two solutions in reversed order of action, and constitutes an *experimentum crucis* in the strict sense of the term.

Electrical excitation of the muscles while immersed in the experimental solutions—in spite of the fact that the induction currents are in large measure short-circuited by the solution—was systematically adopted in preference to excitation of the muscle after the solutions had been run off, because it affords a more complete picture of the gradual effects of such solutions. Currents of sufficient strength are taken to give maximal excitation in spite of the derivation.



Double Myograph to test Action of Substances in Solution.

The diameter of the muscle vessel was slightly less than 3 cm., so that 30 c.c. of fluid gave in it a column about 5 cm. long, more than sufficient to keep the muscle wholly immersed.

The exciting currents passed through the muscle and solution—principally through the former by reason of the copper wires by which it is attached—are taken of such strength as to give assuredly maximal effects. Their direction is not a matter of indifference, the contractions being always unequal to the two directions of excitation; as a rule, but not always, the more effective direction was from tibial to pelvic end, and this was therefore taken as the ordinary direction of exciting currents. But this is not a very essential point, all that is really necessary is to keep to one direction during experiment. Unpolarisable electrodes are also unnecessary, as, indeed, may be readily seen from the records obtained. The magnification of contraction by the lever was × 2.

(From October 9 onwards I used narrower muscle tubes, in order to use up less fluid for each bath, and to have a greater density of current passing through the immersed muscle.)

Alterations of current distribution caused by alterations of resistance of the experimental fluids; the oligodynamic action attributable to the use of copper wire; small differences of room temperature; the possible excitation of intramuscular nerve as well as of the muscle itself, are the principal circumstances that have been considered and recognised to be negligible in the present connection. On the other hand, every care has been taken to secure constant strength of stimulation and constant pressure of the myographic levers against the recording surfaces, which are moved past the levers in tandem by the same clockwork. The influence of considerable differences of temperature was specially examined (*vide infra*).

By preliminary experiments it was found that conveniently graded effects upon muscular excitability were produced by a 5 per 100 solution of alcohol, by a 1 per 100 solution of ether, and by a 1 per 1000 solution of chloroform (by volume in each case). These strengths are of the order of molecular (5·8 c.c. per 100) in the case of alcohol, decimolecular (1·03 per 100) in that of ether, and centimolecular (0·8 per 1000) in that of chloroform.

Thereafter solutions were made up on a molecular scale, taking as the standard of reference a molecular solution of absolute alcohol, and as the first terms of comparison a decimolecular solution of ether and a centimolecular solution of chloroform as tabulated below.

	Sp. gr.	Mol. wt.	c.c. per 100 c.c. saline to give molecular solutions.
Alcohol	0·79	46	5·8
Ether	0·72	74	10·3
Chloroform	1·50	119·5	7·95

Comparisons were systematically made (1) between alcohol and chloroform; (2) between alcohol and ether; and (3) between ether and chloroform. Such comparisons were, whenever possible, made upon the same muscle, preliminary experiments having shown that two or more successive intoxications, if not too profound, by the same strength of solution, are of equal gravity.

The principal indication of the comparative effects of reagents consists in the rate at which the contractility is abolished in solutions (in 0·6 per cent. NaCl in tap water) of various strengths. The rate and amount of return of contractility in 0·6 per cent. NaCl affords confirmatory evidence, of which, however, we have not made systematic use, having done no more than note the facts: (1) that at equal times of immersion the time required for recovery augments with augmented strength of solution, and (2) that at equal strengths of solution the time required for recovery augments with augmented time of immersion.

Comparisons may be established between: the effects of two solutions upon the same muscle successively; or between the effects of two solutions upon two muscles simultaneously; and each kind of comparison has its own obvious advantage and disadvantage. By the method we have adopted of simultaneously recording the contractions of two muscles in series, we secure the advantage of both plans, and minimise the disadvantage of successive comparison by reversing the solutions on the two muscles. Other obvious advantages of the double method are that we get double the number of observations, and that we can readily tell whether an accidental irregularity is due to the stimulus or to the muscle or to the solution.

We may also compare the effects of different solutions upon different muscles, but in such comparisons from muscle to muscle we must take care that the conditions of observation are, as far as possible, identical. We may not, *e.g.*, compare fresh with stale muscle, nor muscles of greatly unequal bulk, nor muscles taken from healthy and unhealthy frogs, nor results obtained at different temperatures. Nevertheless, comparisons of this order are practically available, for under similar conditions the results of experiment with a given solution are closely similar upon different muscles; the "idiosyncrasies" of different muscles are not a very disturbing factor, although, as might be expected, effects are more rapidly produced with very small than with very large muscles.

The two chief fallacies in their order of importance are: (1) a variation in length of the column of fluid, and (2) a considerable variation of temperature.

As regards the column of fluid, it is evident that this must be kept of constant length during an observation, since the fluid forms a derivation circuit surrounding the muscle, which is traversed by only a small fraction of current. The effect of varying the length of column is easily shown by adding or taking away fluid while a series of contractions is in progress. I have, therefore, always been careful to replace fluids by pipette as exactly as possible. If, as has sometimes happened, the difference of excitability and contraction in two muscles has been grossly unequal—even more so than in the case of the pair of muscles used for the record of fig. 1—I have thought it permissible to adjust the tubes in their holders upwards or downwards so as to alter the current lines in suitable degree. But once fixed in position, the tubes must not be shifted again; the level of fluid must be kept unaltered throughout experiment.

Differences of resistance between different fluids are in most cases of little moment, *e.g.* a cubic centimetre of chloroform does not increase the resistance of a litre of saline enough to influence the exciting current traversing the muscle. In some cases, however, differences of resistance may be such as to affect the current distribution and the response of the muscle, *e.g.* a 10-per-cent. solution of alcohol in saline has an appreciably higher resistance than saline alone.

It may be objected to the method that excitation is not restricted to the muscular substance, but includes intramuscular nerve tissues. To meet this objection I compared the effects on fully curarised and on uncurarised muscle, and found that they were indistinguishable. This fact, however, is of little weight, inasmuch as immersion in normal saline is of itself sufficient to remove the effect of curarisation. But, on reflection, the objection itself is of little weight. As is well known, the direct excitability of muscle outlasts its indirect excitability *viâ* nerve; loss of all contractility is of necessity loss of direct excitability, and whatever might be said as to the beginning of an observation, there can be no doubt that at its end we are dealing with muscle and muscle only; even at the beginning of an observation, since we are using a strength of current more than sufficient to excite muscle as well as nerve, the contraction must be by direct muscular excitation; and even if it were not, if it were by indirect excitation at the beginning, the comparative results of, *e.g.* the action of ether and chloroform, would remain acquired. To use as a test object excitation of the nerve of a nerve muscle preparation would, of course, be a different method, by which the tissue specially under investigation would be the end-plate. To use as an index the minimal strength of stimulation giving contraction would again be a different method, by which at first indirect and later direct excitability would be investigated. I have avoided both these proceedings, and have preferred to follow the method described because it is more practicable and less ambiguous

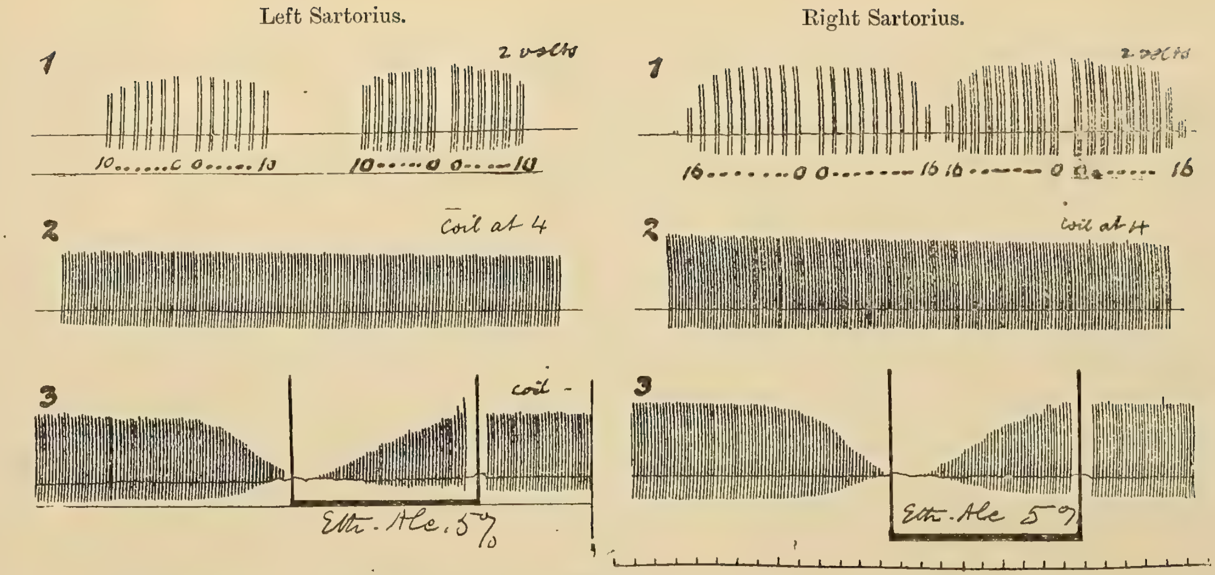


Fig. 1.—September 19, 1908. Temperature = 18°. Simultaneous Records of the Two Sartorius Muscles of a Frog taken on the Double Railway Myograph.

The first pair of records is by groups of contractions with increasing and decreasing strengths of excitation—on the right with the secondary coil at 16, 14, 12...0 ; 0, 2, 4...16 cm. ; on the left at 10, 8, 6...0 ; 0, 2, 4...10 cm.—*i.e.* the apparent excitability happens to be greater on the right than on the left.

The second pair of lines gives the two series of normal contractions by maximal excitations at 10-second intervals with the secondary coil at 4 cm.

The third pair of lines shows the effect on the two muscles of a 5-per-cent. solution of alcohol. The period of immersion is shown by the black lines. The effect of the alcohol on the two sides is equal, although apparently the original condition of excitability is not identical on the two sides ; the illustration has, in point of fact, been chosen for this reason, as it indicates that an identity of excitability need not absolutely be secured. The apparent inequality is principally due to differences of current distribution on the two sides ; it can be corrected by slipping the glass tubes up or down in their holders so that the depth of immersion of the muscle is altered until both muscles respond more nearly alike to a given excitation. The solution in which the muscles are normally immersed is 0·6 per cent. NaCl in tap water.

in its results. I felt justified in making this choice by the results of experiments made many years ago on direct and indirect excitability, and on the junction between nerve and muscle.*

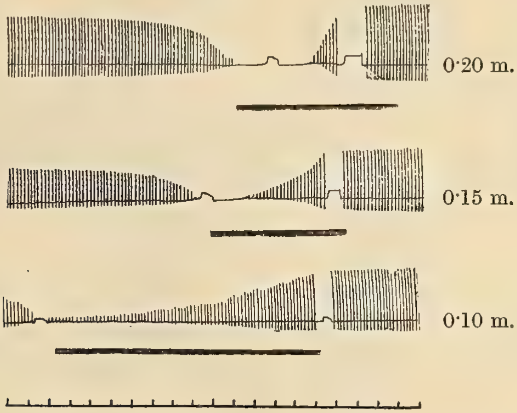


Fig. 2.—August 21, 1908. Effect of Ether Solution at Strengths of 0·1, 0·15 and 0·2 m. (= 1, 1·5 and 2 per cent. by volume).

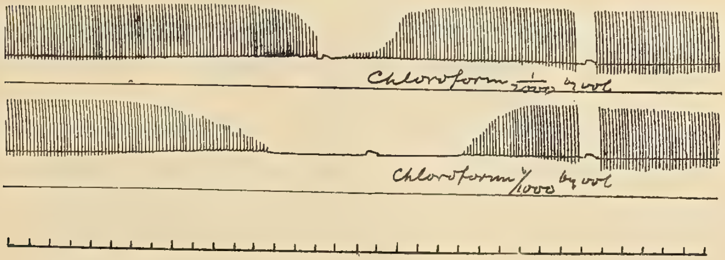


Fig. 3.—August 20, 1908. Comparison between the Effects of Chloroform in 1/1000 and 1/2000 dilution in normal saline (= *m*/80 and *m*/160) and of Ether in 1/100 dilution (= *m*/10).

In chloroform 1/1000 contractility is abolished in 5 minutes.
„ 1/2000 „ „ 12 „

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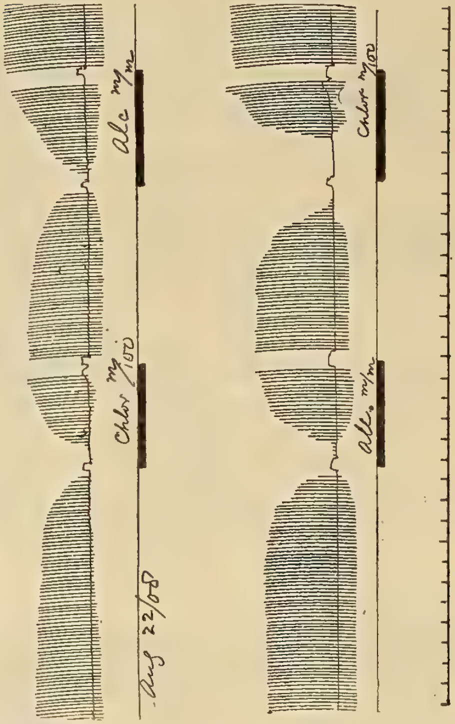
* "Experiments and Observations relating to the Process of Fatigue and Recovery," First Report, 'British Medical Journal,' July, 1885; Second Report, 'British Medical Journal,' July, 1886. In the present connection the principal conclusion of the investigation (carried out during my tenure of a research scholarship to the British Medical Association) was to the effect that the junction between nerve and muscle is functionally a weak link in the neuro-muscular chain, being the first to suffer in its transmitting function in fatigue (by indirect excitation) in intoxications ("curarisation") and in pathological degeneration.

I. *Alcohol* v. *Chloroform.*



Minutes.	Left.	Right.
0 to 5	In normal saline ...	In normal saline.
5 to 10	In alcohol, 1 mol. solution, contractility lost in 4 minutes.	In chloroform, *m*/100 solution, contractility lost in 2½ minutes.
10 to 19	In normal saline ...	In normal saline.
19 to 24	In chloroform, *m*/100 solution, contractility lost in 3 minutes.	In alcohol, 1 mol. solution, contractility lost in 3 minutes.
24 to 34	In normal saline ...	In normal saline.

(Continuation of record not reproduced.)

35 to 40	In alcohol, 1 mol. solution, contractility lost in 5 minutes.	In chloroform, *m*/100 solution, contractility lost in 5 minutes.
40 to 50	In normal saline	In normal saline.
50 to 55	In chloroform, *m*/100 solution, contractility lost in 4 minutes.	In chloroform, *m*/100 solution, contractility lost in 3½ minutes.
55 to 70	In normal saline	In normal saline.
70 to 73	In ether, 0·2 mol. solution, contractility lost in 1 minute.	In chloroform, 0·02 mol. solution contractility lost in 1½ minutes.
73 to 83	In normal saline	In normal saline.
83 to 88	In alcohol, 1 mol. solution, contractility lost in 5 minutes.	In alcohol, 1 mol. solution, contractility lost in 5 minutes.

Fig. 4.—August 22, 1908. Simultaneous Record of Left and Right Sartorius Muscles. Comparative Effects of Ethyl Alcohol (molecular solution in normal saline, 5·8 c.c. per 100) and of Chloroform (centimolecular solution in normal saline, 0·08 c.c. per 100). Temperature = 20°. In the continuation of this experiment, comparisons were made with 0·2 m. ether and 0·02 m. chloroform (not reproduced).

It appears from this experiment that chloroform of centimolecular strength is slightly more effective than alcohol of molecular strength.

From a further experiment made with chloroform at 0·009 m. as compared with alcohol of standard strength, it is found that at this strength chloroform is considerably less effective. The physiological equality of chloroform with our alcohol standard is between 0·010 and 0·009 m., nearer to the former than to the latter value; we have therefore taken as a sufficiently close approximation that

$$\tfrac{1}{100}\,\mathrm{C} = 1\,\mathrm{A} \quad \text{or} \quad 100\,\mathrm{A} = 1\,\mathrm{C}.$$

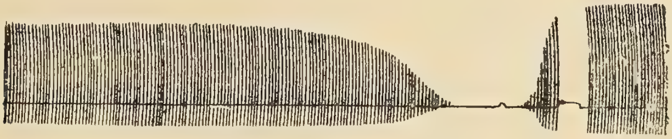
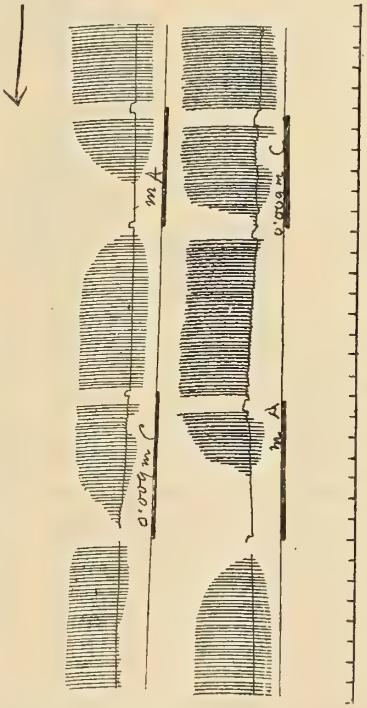


Fig. 5.



Minutes.	Left.	Right.
35 to 40	In normal saline	In normal saline.
36 to 41	In alcohol, 1 mol. solution, contractility lost in 3 minutes.	In chloroform, 0·009 mol. solution, contractility lost in 4½ minutes.
41 to 49	In saline	In saline.
49 to 56	In chloroform, 0·009 mol. solution, contractility lost in 6 minutes.	In alcohol, 1 mol. solution, contractility lost in 3½ minutes.
56 to 63	In saline	In saline.

(Continuation of record not reproduced.)

70 to 75	In saline	In saline.
75 to 82	In chloroform, 0·009 mol. solution, contractility lost in 5 minutes.	In alcohol, 1 mol. solution, contractility lost in 3½ minutes.
82 to 88	In saline	In saline.
88 to 94	In alcohol, 1 mol. solution, contractility lost in 3 minutes.	In chloroform, 0·009 mol. solution, contractility lost in 5 minutes.

Fig. 6.—August 25, 1908. Simultaneous Record of Left and Right Sartorius Muscles. Effects of Ethyl Alcohol, 1 m. solution, and of Chloroform, 0·009 m. solution. Temperature = 20°.

This relation is not confined to this particular strength. In an experiment with 2 m. alcohol and 0·02 m. chloroform, the two reagents produced substantially equal effects (October 6).

II. *Alcohol* v. *Ether*.

Similar considerations apply to the estimation of the relative physiological efficiency of alcohol and ether.

As compared with a molecular solution of ethyl alcohol it was found—

That a 0·1 mol. solution of ether was too weak,
„ 0·2 „ „ too strong,
„ 0·15 „ „ slightly too strong,

and that the closest approximation to equality of effects was obtained with 0·13 and 0·12 mol. solutions, from which it is concluded that molecule for molecule ether is between seven and eight times as powerful as alcohol.

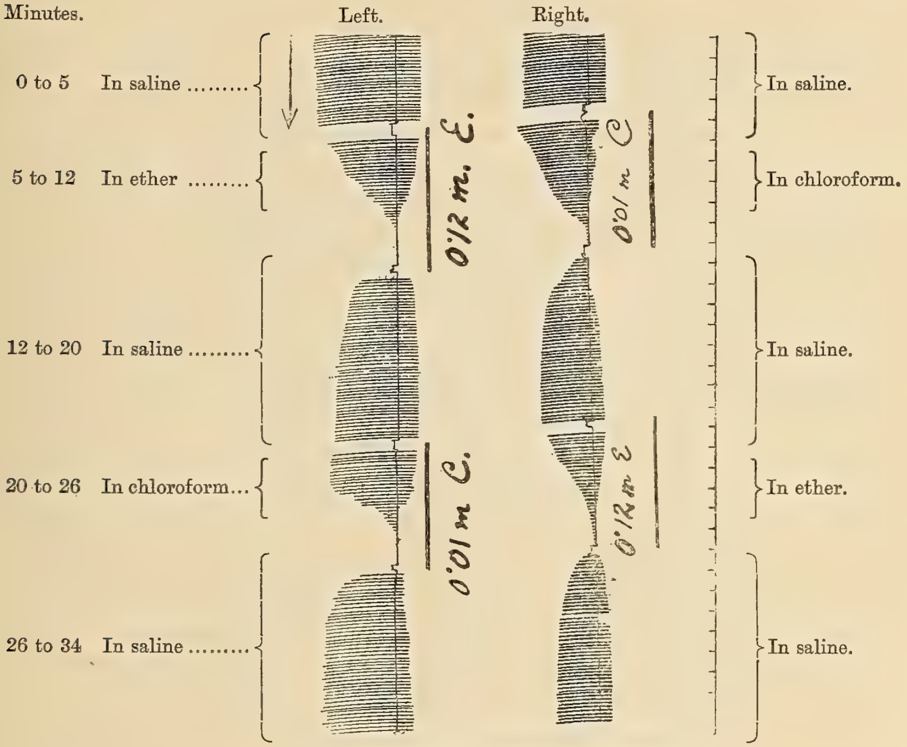
III. *Chloroform* v. *Ether*.

Similar considerations apply to the estimation of the relative physiological efficiency of chloroform and ether.

We commenced by comparing the effects of a 1-per-cent. (by volume) solution of ether and a 1-per-1000 solution of chloroform, these two strengths being respectively equal to 0·1 m. and 0·012 m. The ether solution proved to be considerably the weaker of the two.

For the next trial we took double the strength of ether, viz., 2 per 100 or 0·2 m., and found that at this strength the ether solution was considerably stronger than a chloroform solution of 0·8 per 1000 (= 0·01 m.). In a further comparison between ether, 0·15 m., and chloroform, 0·01 m., the former was still considerably the stronger, and from further trial of 0·13 m. and of 0·12 m. ether we finally determined as nearest to physiological equivalence:

0·01 chloroform = 0·12 ether.



(Continuation of record not reproduced.)

40 to 45	In saline..................................	In saline.
45 to 52	In chloroform, contractility lost in 4½ minutes.	In ether, contractility lost in 6 minutes.
52 to 61	In saline..................................	In saline.
61 to 68	In ether, contractility lost in 4 minutes.	In chloroform, contractility lost in 7 minutes.

FIG. 7.—August 26, 1908. Simultaneous Record of the Effects of Chloroform, 0·01 m., and of Ether, 0·12 m., on Two Sartorius Muscles.

The general conclusion from the foregoing experiments is given in the following tabular summary :—

Physiological Equivalence.

	By molecules.	By weight.	By volume.
Alcohol......................	100	100	100
Ether	12	19·3	21·3
Chloroform	1	2·6	1·4

I.e. 1 molecule chloroform = 12 molecules ether = 100 molecules alcohol. *I.e.* a chloroform molecule is 12 times as powerful as a molecule of ether and 100 times as powerful as a molecule of alcohol.

[By weight approximately, 1 gramme of chloroform = 8 grammes of ether = 40 grammes of alcohol.

By volume approximately, 1 c.c. of chloroform = 15 c.c. of ether = 75 c.c. of alcohol.]

Influence of Temperature upon the Rate of Intoxication.

In my earlier experiments upon the rate of intoxication of muscle by alcohol, ether, and chloroform, I paid no particular attention to the temperature beyond noting that the ordinary room temperature during those experiments was comparatively steady at 19° to 21°. But as the degree of precision of which the method was susceptible became apparent, I undertook to examine the quantitative effect of the temperature factor.

The first experiment in this direction (August 18) was made with a 5-per-cent. solution of ethyl alcohol for the purpose of testing the influence of temperature upon the velocity of the reaction between alcohol and muscle upon which the abolition of contractility depends. At 19° muscular contractility was abolished in 7 minutes; at 30° muscular contractility was abolished in 2½ minutes; the velocity of reaction in this case was augmented in very similar degree to the augmentations with raised temperature observed in the saponification of ethyl acetate and in cases of vegetable activity.* In these cases it has been observed that the velocity is increased between twice and thrice with a rise of 10°; in the case of alcohol and muscle the reaction was accelerated nearly threefold by a rise of temperature of 11°.

Similar results were obtained as regards the effect of raised temperature upon velocity of reaction in the case of chloroform and in that of ether.

In the experiment of August 25, the times of abolition of contractility by a 0·02 mol. solution of chloroform (1·6 c.c. per 1000) were—

At 19°	2 min.	and 2½ min.
28°	0 min. 45 sec.	„ 1½ „

In the experiment of August 27 (fig. 8) the times of abolition of contractility by a 0·15 mol. solution of ether (1·5 c.c. per 100) were—

At 20°	4 min.	and 4½ min.
28°	1½ „	„ 1½ „

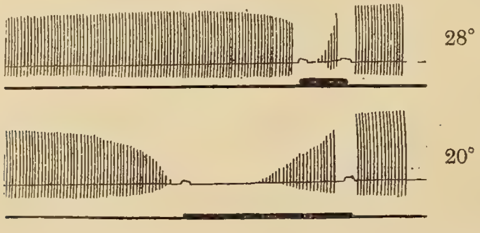


Fig. 8.—August 27, 1908. Effect of Ether Solution, 0·15 m., on a Sartorius Muscle at 20° and at 28°.

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* Cohen, 'Beiträge über Physikalische Chemie,' 1901, pp. 37, 43, and 45.

In an experiment of August 28, in which the times of abolition by a 0·01 mol. solution of chloroform (0·8 c.c. per 1000 c.c. saline) were taken by stop-watch, the numbers were noted as 4 minutes at 20°, 2 minutes at 30°, 1¼ minutes at 37°.

[*Note* (*added September* 1).—In a report recently presented to Section I of the British Association at Winnipeg, I have brought forward evidence to show that effects of the two anæsthetics, chloroform and ether, are simply additive, *i.e.* the sum of their individual effects.

Taking, *e.g.*, a mixture composed of (1 gramme chloroform + 8 grammes ether) per 1000 c.c. saline, I find that the solution is twice as powerful as a solution of 1 gramme $CHCl_3$ per 1000, or 8 grammes Et_2O per 1000.

Taking, as a point of departure, that 1 c.c. $CHCl_3$ is physiologically equivalent to 15 c.c. Et_2O, I find that the saline solution of a mixture composed of equal volumes of chloroform and ether is approximately half as powerful (actually rather more than half) as the saline solution of a volume of chloroform equal to that of the volume of mixture in solution.

Assuming, as before, that 1 c.c. $CHCl_3$ = 15 c.c. Et_2O, I calculate that the physiological power of a mixture used in clinical medicine composed of two volumes $CHCl_3$ and three volumes Et_2O is 0·27 as compared with the power of chloroform taken = 1·00.

Similarly, that the theoretical value of the well-known A.C.E mixture (one volume alcohol + two volumes chloroform + three volumes ether) referred to the same standard is 0·23.

To put these estimates to the test of experiment, a careful comparison was made of three freshly-prepared solutions, containing respectively—

(1) 2·5 c.c. per 1000 of the mixture (2C + 3E).
(2) 1 c.c. per 1000 of chloroform alone.
(3) 2·5 c.c. per 1000 of the mixture (1A + 2C + 3E).

In correspondence with the fact that the theoretical equivalent amount, in the case of the first solution = 2·3 c.c., and in that of the second solution = 2·7 c.c. (as compared with 1 c.c. in the second or standard solution), it was found that the effect of the first solution came out slightly above that of the standard solution, while that of the third solution came out slightly below that of the standard.]

Note.—Dr. Veley has been kind enough to give me the following calculation, from which it appears that we are really dealing with an alteration of reaction velocity:—

At a temperature of 20° a time 4 min. was required for the abolition of contractility.
„ 30° „ 2 „ „ „ „
„ 37° „ 1·25 „ „ „ „

Hence at the end of each minute,

$$\left.\begin{array}{llll} \text{in Case} & \text{(I)} \ldots\ldots & 0{\cdot}25 & \text{unit change took place} \\ \text{,,} & \text{(II)} \ldots\ldots & 0{\cdot}5 & \text{,,} \quad \text{,,} \\ \text{,,} & \text{(III)} \ldots\ldots & 0{\cdot}6 & \text{,,} \quad \text{,,} \end{array}\right\} \begin{array}{l} \text{Referring to unity,} \\ \text{ratio of numbers} \\ = 1:2:3{\cdot}4. \end{array}$$

By Esson's formula,* $(\kappa_1 - \kappa) = m \log(\tau_1 - \tau)$.

$$\begin{array}{rcr@{\qquad\qquad}rcr} \log 2 & = & 3010 & \log 3{\cdot}4 & = & 5051 \\ \log 1 & & & & & \\ \hline & & 3010 & & & 5051 \end{array}$$

$$\begin{array}{rcr@{\qquad\qquad}rcr} \log 273+30 & = & 4814 & \log 273+37 & = & 4914 \\ \log 273+20 & = & 4669 & \log 273+20 & = & 4669 \\ \hline & & 0145 & & & 0245 \end{array}$$

$$\frac{3010}{0145} = 20{\cdot}8. \qquad \frac{5051}{245} = 20{\cdot}6. \qquad \text{Mean, } 20{\cdot}7.$$

Hence $0{\cdot}0145 \times 20{\cdot}7 = 0{\cdot}3002 = 1{\cdot}99$ calc., $2{\cdot}00$ found.
$0{\cdot}0245 \times 20{\cdot}7 = 0{\cdot}5072 = 3{\cdot}22$ „ $3{\cdot}20$ „

The graph of $\log \kappa'/\kappa$ in terms of $\log \tau/\tau$ is a straight line and is the most convenient form of representation.

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* 'Phil. Trans.,' A, 1895, vol. 186, p. 861.

Studies on the Structure and Affinities of Cretaceous Plants.

By MARIE C. STOPES, Ph.D., D.Sc., F.L.S., Lecturer in Palæobotany, Manchester University, and K. FUJII, Ph.D., Assistant Professor of Botany, Imperial University, Tokio.

(Communicated by Dr. D. H. Scott, F.R.S. Received May 13,—Read May 27, 1909.)

(Abstract.)

The authors comment on the importance of the work done on the flora of the Palæozoic period, and the botanical interest that would attach to similar petrifactions of plants from all ages of the Mesozoic period. They have had the good fortune to find excellently preserved material from the Cretaceous of Northern Japan.

In the present paper they describe 18 plants from this material, which is extraordinarily rich. As hitherto there has been very little known from anatomical material of plants of this age, the present paper is by no means final, but is in the nature of a pioneer chart of the ground.

The petrifaction of the cells of the plants is often extremely good, though the fragments are not so complete as could be desired. The plant structures include stems, roots, leaves, cones, fern sporangia, and even an Angiospermic flower, the first petrifaction of a flower to be described. The *débris* lie together in the nodules in much the same way that the *débris* lie in the Coal-balls of the Palæozoic, though they are mixed with fragments of shells. The latter are largely Ammonites and serve to determine the age of the petrifactions.

The flora as a whole represents an interesting mixed flora such as has not hitherto come to light among petrifactions.

Roughly speaking, the flora seems to have consisted of about one-third Angiosperms, slightly more than one-third Gymnosperms, and the rest of ferns and lower plants. The anatomy of the early Angiosperms being such a desideratum in botany, their presence in the petrifactions renders them doubly interesting, and particularly when they are found in so evenly balanced a mixed flora.

All the specimens described in this paper were cut in Tokio in the botanical department by the authors.

The plants described are as follows:—

Petrosphæria japonica, gen. et spec. nov. A fungus which has numerous microsclerotia, in the periderm of one of the Angiosperms, *Saururopsis.*

Schizæopteris Tansleii, gen. et spec. nov. The sorus and sporangia of a Schizæaceous fern.

Fasciostelopteris mesozoica, gen. et spec. nov. The stem and petiole of a fern with a dictyostelic anatomy. Probably allied to the *Dicksoniaceæ*.

Fern rootlets, in excellent state of preservation, showing the diarch stele of the leptosporangiate ferns.

Zamiophyllum cordaitiforme, gen. et spec. nov. The leaf of what appears to be some plant of Cycadean affinity, the anatomy bearing considerable resemblance to that of *Cordaites*.

Yezonia vulgaris, gen. et spec. nov. A Gymnosperm, of which stems, unthickened twigs, leafy axes, are all very plentiful. It is the commonest plant in the material, and at the same time the most unique. In the anatomy of both main axis and foliage it is not like any known type.

Yezostrobus Oliverii, gen. et spec. nov. The fructification of a Gymnosperm, the cone bearing simple scales with seeds, one on each, which are like those of Cycads in some respects, but have a nucellus standing up entirely free from the integument with a well marked epidermis between.

Though continuity is lacking between these two plants, there seems considerable ground for suspecting them of belonging to the same plant from anatomical points of likeness.

Araucarioxylon tankoensis, spec. nov. Secondary wood, showing remarkably clear pittings in the transverse sections.

Cedroxylon Matsumurii, spec. nov. Well preserved secondary wood.

Cedroxylon Yendoii, spec. nov. Secondary wood, with traumatic resin canals.

Cunninghamiostrobus yubariensis, gen. et spec. nov. A cone, as its name implies, belonging to the family of the Cunninghamias, with its external appearance partly preserved and the cone scales and axis fairly well petrified. The seeds have apparently been scattered.

Cryptomeriopsis antiqua, gen. et spec. nov. Stem with leaves attached, the foliage very like that of a *Cryptomeria*.

Sauruopsis niponensis, gen. et spec. nov. The stem and attached roots of an Angiosperm, probably to be included in the Saururaceæ.

Jugloxylon Hamaoanum, gen. et spec. nov. The secondary wood of an Angiosperm.

Populocaulis yezoensis, gen. et spec. nov. The stems of an Angiosperm, with cortical tissue.

Fagoxylon hokkaidense, gen. et spec. nov. The secondary wood of an Angiosperm.

Sabiocaulis Sakurii, gen. et spec. nov. Minute stems, and older twigs of an Angiosperm, with cortex, and well preserved and characteristic anatomy.

Cretovarium japonicum, gen. et spec. nov. The flower of an Angiosperm, of which there are several specimens.

Of this list of plants, the commonest, *i.e.* those which have yielded the greatest number of specimens in the course of the work, are *Yezonia, Sabiocaulis,* and *Cretovarium.* It is noteworthy that these are among the most unusual and the most interesting of the plants.

The authors acknowledge much assistance in the work from the Royal Society Government Grant Committee, which made it possible for one of them (M. C. S.) to attempt the work; and from the various departments of the Imperial Government of Japan in the course of collecting and preparing the material.

OBITUARY NOTICES

OF

FELLOWS DECEASED.

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DAVID JAMES HAMILTON, 1849—1909.

After protracted illness, the death of Prof. David James Hamilton, M.B., LL.D., F.R.S., F.R.S.E., F.R.C.S.E., took place at his residence in Aberdeen on February 19, 1909.

The subject of this memoir was born sixty years ago at Falkirk. He received his medical education at Edinburgh University, from which he graduated as M.B. in 1878. He subsequently held several clinical appointments: two in Edinburgh, where he acted as House Surgeon in the Royal Infirmary, then as Resident in the Chalmers Hospital; a third in Liverpool, where he held a Resident Surgeoncy in the Northern Hospital. Even at this early period of his career he was strongly attracted towards pathological problems, for the study of which his thorough acquaintance with the physiology of that period formed an all-important basis. This predilection for a branch of medical science at that time much neglected, was happily confirmed when the Triennial Astley Cooper Prize was awarded him for a thesis dealing with "The Diseases and Injuries of the Spinal Cord," in which the result of much careful observation and research was embodied.

This mark of appreciation effectually determined Hamilton's path in life, and in order to acquaint himself more thoroughly with the thought and technique of other schools, he proceeded to the Continent, visiting Strassburg, Munich, Vienna, and Paris in succession. After thus spending two years in close contact with the leading European pathologists of the time, he returned home in 1875 on his appointment as Assistant to the late Prof. Sanders in Edinburgh University. Somewhat later he became pathologist to the Royal Infirmary, a post which furnished him with ample facilities for the prosecution of observation and research. At this time he organised a course on morbid histology, which was largely attended, mainly by young graduates, who were not slow in recognising the vigour, resource, and thoroughness of their teacher.

An even wider opportunity for studying the most effective methods of class instruction presented itself in the winter of 1880–1, when, owing to the ill-health of Prof. Sanders, necessitating the appointment of a temporary substitute, Hamilton found himself entrusted with the entire duties—educational and administrative—of the University Pathological Department.

Before the endowment of the Erasmus Wilson Chair in 1882, the University of Aberdeen had no teacher in pathology apart from the professors of Practice of Medicine and of Surgery, who did what the discharge of their more obvious duties permitted to remedy the deficiency. On his election as the first occupant of the Chair of Pathology, Hamilton naturally found that his resources were limited; it may be said that they were non-existent, for he inherited no department and but the scantiest equipment. But his enthusiasm and determination of purpose, coupled with exceptional physical vigour, fully qualified him to deal with the difficult position, so that in a surprisingly short space of time he was presiding over classes of systematic and practical study in which his able and inspiring teaching was illustrated by a wealth of material, amongst which his models of pathological conditions, moulded and coloured to perfection, were marvellous counterfeits of reality. Practically all these accessories were prepared either by his own hands or under his direct supervision. Though his most pressing needs were satisfied for the moment, others speedily presented themselves. With the active development in bacteriology then proceeding, the requirements of pathological departments demanded an accelerated expansion; more experimental and teaching accommodation had to be found, apparatus to satisfy the needs of an elaborate technique to be procured, but by unfaltering effort Hamilton seldom failed in obtaining that which he deemed essential in order to keep his laboratories fully abreast of the requirements of the time. It would, in fact, have been almost impossible for those who presided over the University's coffer to have rejected the vigorous and conclusive appeals which he made to their liberality.

Hamilton's exceptional qualifications as a teacher were speedily recognised. They were endowments rather than acquirements. Some scientists with high versatility in observation and investigation possess a less conspicuous qualification for imparting their enthusiasm and information to others, but he was a born teacher whose clear incisive style, carefully ordered facts, and closely argued theories compelled the attention and conviction of his hearers. By some he has been styled dogmatic, but if he sometimes pronounced a decided judgment on a contested point, it was always preceded by a fair statement of the observations and theories of others. That his own views were decidedly and vigorously instilled, was no doubt a factor in the success of his method of instruction, in so far that the student was left with a perfectly clear conception of the standpoint adopted by his master.

Whilst his duties at Marischal College and at the Royal Aberdeen Infirmary (in which institution in his capacity as pathologist he had entirely remodelled the *post-mortem* department) occupied his time very fully, Hamilton was devoting much of his evenings to preparing his 'Text Book of Pathology' for publication. On its appearance in 1889, the book was received in most quarters with unqualified approval. It was recognised as being a thorough and comprehensive work of reference in the various aspects of pathological study, based on the teachings of physiology to ensure a due comprehension of morbid function, minute, clear, and practical in its details of conditions and methods, illustrated with an exactitude and skill unattainable except by those possessing not merely the requisite scientific knowledge, but an artistic temperament to perfect the delineation.

That all critics should be entirely satisfied with the work was naturally impossible: too little attention paid to the vital manifestations of morbidity, too much to structural abnormality, the experimental aspect of study unduly subordinated, these were amongst the strictures; but when the critic had sufficiently vindicated his own standpoint, he usually showed himself constrained to praise much of the text and all of its illustrations. It was no small assistance to Hamilton in this literary work that his earlier investigations had brought him into the closest contact with very various questions of a pathological character; he had not limited himself to a narrow field of observation, but had been truly catholic in his selection of objects of study. A recital of some of his chief contributions to pathology may illustrate this point.

In 1879 he commenced a series of very capable articles dealing with various morbid conditions of the lung. The papers appeared at brief intervals in the issues of 'The Practitioner' throughout a period of two years, so that the name of the author became familiar to medical man and specialist alike. (It may be mentioned that Hamilton particularly desired to accentuate the value of pathological study in its proper relationship to diagnosis and treatment.) Topographical knowledge of the central nervous system was enriched by his contributions on the conducting paths in the brain, and especially by his work on the corpus callosum in adult and embryonic conditions. (In prosecuting these researches he was assisted by a grant from the Royal Society.) In 1882 a communication was made by him to the Royal Society of Edinburgh, in which he dealt with a physical explanation of diapedesis, and illustrated his theories by novel and ingenious mechanism.

Much originality of idea, expressed with his wonted lucidity, is displayed in his studies relating to sponge grafting, and to embolic infarction, as well as in articles bearing upon such matters as the influence of heredity in disease, the pathology of gastric dyspepsia, and the alimentary canal as a source of contagion.

Hamilton showed a keen interest in the pathological conditions occurring amongst domestic animals, especially those which contribute to the food supply of man, and his later work in this direction is important, not merely from its bearing upon agricultural economics, but also as a practical addition to current knowledge of invasion and resistance. His inquiry into the relationship of human to bovine tuberculosis strengthened by its results his opposition to the views adopted by Koch; whilst his laborious investigation into the etiology, symptomatology, and prophylaxis of certain disorders occurring amongst sheep led to very interesting and valuable conclusions.

This—his last, and probably his most important work—must be referred to in some detail. The commencement of this inquiry is now remote, for it was in 1881 that Hamilton was sent to the Island of Skye by the Highland and Agricultural Society as the expert member of a committee charged with investigation of the disease known as "Braxy" (*morbus subitarius ovis*). The time of year chosen for the expedition proved unfavourable for this purpose. It was not until 1897 that Hamilton found himself able to resume the research: in that year he visited the Fort William district, with other localities where disease was rampant, observing the symptoms and topography not only of braxy but of other members of the group of disorders with which it is frequently confounded by stock-masters and shepherds. From year to year these excursions to various infected areas were repeated with a similar object, and as their result, augmented by official information mainly derived from Ireland, he was able to settle and map out the topography of braxy. But this was merely a preliminary to the much more important work which was instituted by the Departmental Committee appointed by the Board of Agriculture in 1901, upon which Hamilton acted as chairman and expert. Plans of action were agreed upon and observation stations organised in localities where disease occurred endemically amongst sheep, the chief being located at Kielder, in Northumberland. The report of this Committee appeared three years ago in the form of a Blue book. It deals chiefly with braxy and louping-ill, though touching upon several other disorders which are held to be individually distinct. Hamilton fully confirmed the description of the braxy micro-organism, first recognised by Ivar Nielsen in 1888, and further, added several important observations relating to its seasonal activity and manner of invasion, together with a suggested method of prophylaxis. The louping-ill disease (*chorea paralytica ovis*) which had been a veritable mystery, was finally unravelled, the bacillus found, its characteristics under cultivation studied, its manner of development and invasion traced, its seasonal activity explained, a plan for protection against it elaborated and tested with most encouraging results. The pages of the report testify to the prolonged and arduous nature of a research which, step by step, led the way to an entirely satisfactory and practical issue. Apart from the information they contain bearing upon seasonal receptivity towards infection, the nature of intestinal infection, the duality of symptoms produced by the toxine originated by the same micro-organism, together with other matters which may have their signification for man as well as for the animals which Hamilton observed, the work has a direct bearing upon agriculture of a far reaching character. The need for similar investigation into other disorders of the sheep, which are touched upon incidentally in the report, is indicated; it was, indeed, Hamilton's intention to make a thorough study of these individually, but their elucidation was not to be at his hands.

The attachment of his former pupils was suitably shown in 1906, when, on the completion of his twenty-fifth year of service in the University, a volume of 'Studies in Pathology' was prepared in his honour, to which many old students, now professors and lecturers in other schools, were contributors. His jubilee coincided in point of time with the celebration of the quatercentenary of Aberdeen University. He appeared to be at that time in the enjoyment of his accustomed vigour, but not long afterwards symptoms of a disquieting character became apparent: exertion fatigued him, vigour of step and speech were less manifest, and although he only relinquished his work at intervals under pressing medical advice, it was evident that he was losing ground. By the end of 1908 he was confined to his room and unable to be present at the funeral of his wife when her decease occurred somewhat later.

Under the influence of this bereavement and the progress of his malady, Hamilton's condition became steadily worse; he recognised the impossibility of recovery and resigned his Chair; five months later, in the spring of 1909, death terminated his suffering.

As we have endeavoured to summarise the work of a lifetime, it is fitting that we should glance for a moment at the outstanding characteristics of the man who accomplished it. Hamilton possessed a strongly defined individuality, intense and ardent, firm in upholding a conviction, direct and enthusiastic in supporting it. Finesse, compromise, and ambiguity were alike foreign to his nature; if he was frank and outspoken he was incapable of harbouring feelings of bitterness or resentment towards others who held different views. To his intimate friends there was a perennial freshness and geniality in the relationship: his keen sense of humour, interpreted by the expressive grey eyes and the musical infectious laugh, added to the charm of his company. Beyond his work he had wide interests and deep sources of pleasure; he loved nature and keenly appreciated the artistic, whether in form or colour. On several occasions he lectured on artistic themes, architecture included, always exhibiting a fine enthusiasm for work which he recognised as harmonious and genuine. In music he was a connoisseur and was himself possessed of a melodious voice.

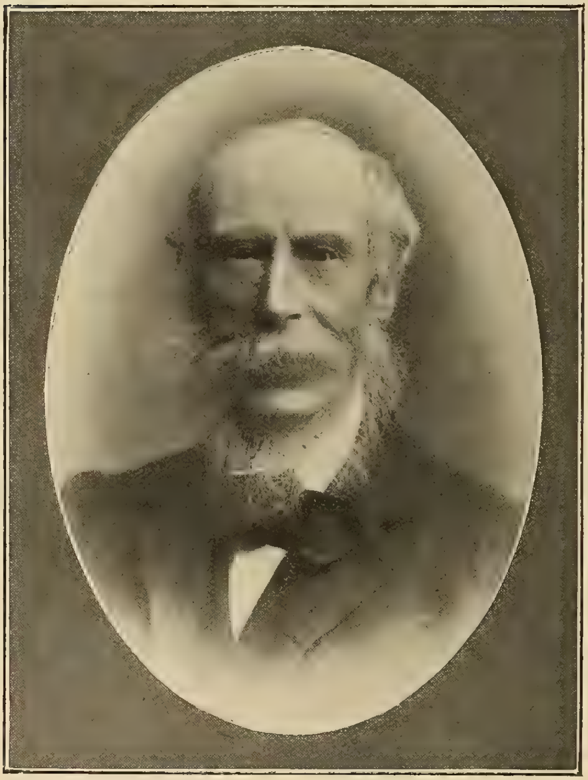
Hamilton was a member of many learned societies and bodies; his election to the Fellowship of the Royal Society in 1908 and his laureation as LL.D. by his Alma Mater soon after, were tokens of appreciation which caused him peculiar gratification. Had he lived to the spring graduation of the current year he would have received a similar recognition from the University in which he had served with much distinction throughout a period of twenty-six years.

Hamilton was twice married: his first wife died seventeen years ago; his second, a daughter of Mr. John Wilson, of Falkirk, predeceased him by a few months.

J. T. C.

WILFRID HUDLESTON HUDLESTON, 1828—1909.

In the history of Geological Science two classes of individuals have, at various times, contributed to its advancement, namely, the amateur investigator and the professional worker. Of these the amateurs were certainly amongst the earliest in the field, and indeed it may be truly said that, but for their labours, the initiation of the Geological Society and the Survey itself as a public department would scarcely have met with so early a reception in this country.



Thanks to our universities and public schools, well-trained professional workers have now become so numerous that there seems little room left for the amateur; nevertheless, so fascinating is the science that geology is still pursued with marked success by many private persons, purely *con amore* and often as a leisure hour pursuit or an agreeable concomitant of travel. Foremost amongst those non-professional geologists, who devoted his life for many years to this science, must be placed the name of Wilfrid Hudleston Hudleston (formerly Simpson).

Born at York, June 2, 1828, Wilfrid Hudleston Simpson was the eldest son of Dr. John Simpson, of Knaresborough, and is, on his father's side, a descendant of three generations of Yorkshire "medicine-men." His mother, *née* Elizabeth Ward, was heiress of the Hudlestons of Cumberland, and in 1867, on succeeding to the family estates, Wilfrid, by letters patent, assumed the name of Hudleston, by which he is best known among geologists.

From 1831 to 1834 he resided with his parents in Harrogate, where his first playfellow was Henry Clifton Sorby—who afterwards became so distinguished a geologist and a President of the Geological Society of London.

Young Simpson received his early education at St. Peter's School, York, from which he was transferred to Uppingham School, and subsequently entered St. John's College, Cambridge, where he graduated B.A. in 1850. Up to this time, as a school-boy and an undergraduate, Wilfrid had evinced no special predilection for geology. In his last term he attended Sedgwick's lectures and was much impressed with the manner and appearance of that distinguished geologist. His earliest scientific pursuit was ornithology, commenced at fifteen years of age whilst still at Uppingham School. At Cambridge he was associated with Alfred Newton from 1848 and with J. E. Law, making many expeditions in Northumberland, Cumberland, and N.W. Ireland. Afterwards he visited the Norwegian coast and here he became acquainted with John Wolley (author of 'Ootheca Wolleyana'), and with him he collected in Finland and Lapland, also with Alfred Newton and John Wolley in 1855 and in 1856 in the Island of Öland and Sweden.

In 1857 he joined Canon Tristram and Osbert Salvin in an ornithological expedition to Algeria. Together they explored the Eastern Atlas, visiting Tunis, Constantine, Kef, etc. The years 1859—60 were chiefly spent by Wilfrid Simpson in Greece and part of Turkey (the Dobrudscha, now Roumania). His last ornithological trip was made in 1861 to Switzerland.

On leaving Cambridge he devoted some time to the study of the Law and was called to the Bar in 1853, but never practised.

From 1862 to 1867 Mr. Wilfrid Simpson held a commission in the Kent Artillery (Militia), and performed yearly garrison duties at Dover Castle. About this time he also began a special course of scientific studies, selecting more particularly Natural History and Chemistry. He studied in Edinburgh under Playfair and Stephenson Macadam, and subsequently, for three sessions, at the Royal College of Chemistry in London under Hoffmann, Frankland, and Valentine. At that time he was uncertain whether to take chemistry or geology as his main subject of pursuit, when an accident decided in favour of the latter. In 1866 he met Marshall Hall at Chamounix and on their return to England he was speedily introduced to many persons interested in geological science, of whom Prof. Morris may be regarded as the chief.

Prof. Morris had a wonderful influence over his pupils and associates, and this was just the attraction which Mr. Simpson, who had now (1867) assumed the patronymic of Hudleston, required to enlist him as a geological recruit, and in due course to make him a "knight of the hammer" for the rest of his life. A close friendship was thus formed which was only terminated by the death of Morris in 1886.

In 1867 he was elected a Fellow of the Geological Society, and in 1871 a Member of the Geologists' Association. Of this Association he became Secretary in 1874, and during the three years of office he spent much time in preparing reports on the various districts visited, some of which are of considerable extent and importance. In 1872 he published his first original paper (with Mr. F. G. H. Price) "On Excavations at the New Law Courts."

His papers on "The Yorkshire Oolites" (1873—78) and "The Corallian

Rocks of England" (with Prof. J. F. Blake (1877)) soon established his reputation as one of our leading geologists.

Mr. Hudleston was elected President of the Geologists' Association in 1881, and his great services rendered to that body during so many years were marked, in March, 1892, by the presentation of an illuminated address.

From 1886 to 1890 he filled the post of Secretary to the Geological Society of London and, in succession to Sir A. Geikie, he was elected President (1892—94). In these years, in addition to his official duties both in connection with the Geological Society and the Geologists' Association, Mr. Hudleston proved himself an able and prolific contributor to the literature of geology. Some twenty papers were written by him on the field-geology of various districts, eight on Chemical Geology, for which he had always a strong predilection, dealt with the Lizard Rocks; the Gneiss Rocks of the N.W. Highlands; Sterry Hunt's Chemical Essays; King and Rowney's views on *Eozoon Canadense*; the Diamond-rock of S. Africa; and Sterry Hunt's views on Serpentines. In Palæontology he published Monographs on the Corallian Gasteropoda of Yorkshire (1880—81). The Gasteropoda of the Portland Rocks (1881) and of the Oxfordian and Lower Oolites of Yorkshire (1882—85), the Gasteropoda of the Inferior Oolite (1887—96), this latter comprising 514 quarto pages of letterpress and 44 quarto plates of fossils; a Catalogue of British Jurassic Gasteropoda (with Mr. E. Wilson), and papers on the Fossils of Western Australia and South Australia.

To this period must also be added Presidential Addresses to the Geologists' Association "On Deep-Sea Investigation" (1881), on the Geology of Palestine (1882, with additional notes in 1885), to the Malton Field Naturalists' Society (1884), and the Devonshire Association (1889); lastly, two Presidential Addresses to the Geological Society (1893—94), and one, later, as President of Section (C) Geology, British Association, Bristol (1898).

On the death of his old friend, Prof. Morris, in 1886, Mr. Hudleston succeeded him as one of the editors of the 'Geological Magazine,' to which journal, since 1879, he had been a frequent contributor, and continued so until his death in the present year. He was a keen student of recent and fossil mollusca and one of the founders of the Malacological Society.

In 1886, accompanied by Dr. Henry Woodward, F.R.S., and Mr. C. E. Robinson, M.Inst.C.E., he carried out some experimental dredgings, with a Brixham trawler and her crew, along the English Channel, and in and near Torbay, in order to study marine mollusca and observe their living habitats. In the following year he engaged a Grimsby steam trawler and her crew, and accompanied by Mr. C. E. Robinson and the late Martin F. Woodward, of the Royal College of Science, he spent three weeks in a dredging cruise in the English Channel and along the French coast.

Mr. Hudleston resided for many years in Cheyne Walk, Chelsea, but removed in 1883 to Oatlands Park, Surrey. This suburban residence, however, interfered with his scientific engagements, and he again took up a residence in town at 8, Stanhope Gardens, South Kensington.

In 1890, he married Miss Rose Benson, second daughter of the late William Heywood Benson, Esq., of Littlethorpe, near Ripon.

Early in 1895, Mr. Hudleston, accompanied by his wife and his friend, Prof. J. F. Blake, F.G.S., left London for Bombay. After leaving Prof. Blake duly installed as Organising Curator of the Museum at Baroda, to which he had just been appointed, Mr. Hudleston journeyed onwards towards the north-west frontier of India. The geological results of this expedition are embodied in "Notes on Indian Geology," read before the Geologists' Association in December, 1895 (see 'Proc. Geol. Assoc.,' vol. 14, p. 226, 1896).

He presided over or took part in the Councils of numerous scientific societies. He was elected, in 1889, President of the Devonshire Association for the Advancement of Science, the Yorkshire Naturalists' Union, and the Malton Field Naturalists' Society; and had been for years a Vice-President of the Dorset Natural History Field Club. He also served as a Member of Council of the Royal Geographical Society, and as President of the Geological Section of the British Association at Bristol in 1898.

In 1897, Mr. Hudleston was awarded the highest honour which the Council of the Geological Society could bestow, namely, the "Wollaston Gold Medal," in recognition of his valuable contributions to our knowledge, including chemical, mineralogical, palæontological, and stratigraphical geology. Special reference was made by the President, Dr. Henry Hicks, F.R.S., to his monograph on "The Inferior Oolite Gasteropoda," contained in the volumes of the Palæontographical Society, which, with the services of four collectors in the field and in cleaning, developing, etc., occupied a period of over twenty years, the descriptions filling 514 quarto pages of letterpress and 44 quarto plates of figures. This fine collection of types has, since the death of Mr. Hudleston, been transferred, as a gift, to the Sedgwick Memorial Museum, Cambridge.

Two later papers deserve special mention, namely, the investigation of the structure of "Creechbarrow in Purbeck" ('Geol. Mag.,' 1902—3), and that "On the Origin of the Marine (Halolimnic) Fauna of Lake Tanganyika" ('Geol. Mag.,' 1904).

In his earlier years, before he became known as a geologist, he took a keen interest in ornithology, and was instrumental in founding, in 1858, in conjunction with the late Prof. Alfred Newton, of Cambridge, Mr. John Wolley, and others, the British Ornithologists' Union; and so lately as December 9, 1908, they commemorated the Society's fiftieth anniversary. To mark the occasion, the Society presented a gold medal to each of the four surviving original members, of whom Mr. Hudleston was one.

In connection with the Armstrong College, Newcastle (in the University of Durham), Mr. Hudleston provided the site and advanced capital for erecting a Marine Biological Laboratory at Cullercoats, Northumberland, to be named the "Dove Laboratory" (after a great ancestor of his family, Eleanor Dove).

It was erected and equipped at a cost of £4000, and was opened in July, 1908, by the Duke of Northumberland. The building is suitably provided with a fine aquarium with numerous tanks, and a large room on the ground floor where experiments on pisciculture may be conducted under improved conditions. It has, in addition, a library, a lecture room, workrooms, etc. The Director is Prof. Meek, M.Sc., under whose direction the entire equipment of the building was carried out.

Mr. Hudleston was a Justice of the Peace for the West Riding of Yorkshire and for East Dorset. He purchased the East Stoke Estate in 1897, for the sake of the shooting, having all his life been a keen sportsman. His latter years were divided between West Holme, Wareham, and his town residence, 8, Stanhope Gardens. He died at West Holme, January 29, 1909, in his eighty-first year.

Mr. Hudleston's life was marked by untiring energy, directed with a steady purpose throughout. As a man of science, may be mentioned the numerous offices he held in connection with the Geological Society, the Geologists' Association, and many other bodies. No fewer than 58 memoirs and papers, extending over a period of 32 years, attest to his energy and ability.

As an ornithologist and a traveller he accomplished much. During his sojourn in the East he acquired a fluent knowledge of modern Greek as well as Arabic. As a magistrate and a landed proprietor he was always earnestly desirous to fulfil his duties; while as a sportsman, both with gun and rod, he exhibited the same keenness as with his geological hammer or in his chemical laboratory and museum.

For many years Wilfrid Hudleston lived much alone, having but a small number of intimate friends: Prof. Morris, F. G. Hilton Price, Henry Woodward, H. W. Monckton, and some few others. Hence the social side of his life was never fully developed. But his earliest ornithological friendships for Prof. Alfred Newton, John Wolley, O. Salvin, Canon Tristram, and J. E. Law remained the strongest and warmest throughout his life, and were only separated by death and as he drifted apart from them in his later geological pursuits.

H. W.

SIR GEORGE KING, 1840—1909.

George King, the only son of Robert King and Cecilia Anderson, was born at Peterhead, where his father was a bookseller and his maternal grandfather was Collector of Inland Revenue, on April 12, 1840. While King was still a child his father moved to Aberdeen, and joined in partnership his elder brother George, a bookseller in that city, where another brother, Arthur, founder of the Aberdeen University Press, was a printer. Partly in conjunction with this press, the firm of G. and R. King developed a publishing business which rendered good service to the north-east of Scotland at a time when difficulties of communication delayed supplies of literature from London, or even from Edinburgh. The partners were members of a family of Independents, a denomination never numerous in Scotland. Both were men of strong character and much ability, and took an active part in promoting the interests of the Congregational community. The senior partner, George, belonged to the Scottish Society of Antiquaries, and was one of the three founders of a still flourishing local Liberal newspaper.* He was for forty years closely connected with the administration in Aberdeen of the Scottish poor-law, and published an essay on "Modern Pauperism" which attracted the attention of social statesmen fifty years ago. He also wrote an historical review of the origin and condition of the Congregational churches in Aberdeenshire and Banffshire, in which, while holding the views of an Independent as regards the organisation of the Anglican Church, he found the Presbyterian form of church government equally unjustifiable on historical grounds. The junior partner, father of the subject of this notice, was the author of a meritorious historical work, 'The Covenanters in the North,' published in 1846, shortly after his death from phthisis, at the early age of thirty-six, in November, 1845. As an Independent, the author was able to handle his subject with sympathy, and at the same time without the bias so often apparent in Presbyterian writings. King's mother died, also of phthisis, at the age of forty, in June, 1850.

Left an orphan at ten, King became the ward of his uncle George, who in the autumn of 1850 transferred him from the preparatory academy he had hitherto attended, to the Aberdeen Grammar School, then under the rectorship of Dr. Melvin, one of the foremost classical schools in Scotland. Two pupils named King, were already there, one in the fifth, another in the second class; the subject of this notice on entering the first class, taught by Mr. (afterwards Sir William) Geddes, was therefore, to the masters, King "tertius," to his schoolmates "Tertius" for short; this agnomen stuck to him till his undergraduate days. At school "Tertius," in spite of constitutional delicacy, was an apt pupil; his guardian, partly on this account, partly because of the boy's poor health, decided to train him to be his successor in the firm, and effect was given to this design when King left school in 1854. The experiment did not succeed as his guardian had hoped, though it cannot be said that this effort to determine King's career was altogether thrown away.

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* Arthur King, of the University Press, also engaged in newspaper enterprise, and became proprietor of the first penny paper published in the north of Scotland.

Contemporaries of King who survive recall the proceedings of a literary society connected with the church to which his uncle belonged. Young King, while in business, was a leading member; his contributions to the discussions, we are told, showed erudition and insight beyond his years. The society subscribed for papers then rarely seen in Scotland; among them the 'Saturday Review,' for which King developed an especial liking that, except when the paper was edited by F. Harris, he retained to the last. It may be noted that when, later in life, King left the Independent community, he transferred his allegiance to the Anglican, not the Calvinist, Church. Not improbably King's sympathies with literature and art, and his practical outlook on life, may have been paternal inheritances. But it is clear that this association with a bookselling and publishing business aided in developing his keen and sure taste, so appreciated by intimate friends, so unsuspected by others. Nor can it be doubted that his instinct for the essential, his mastery of complex detail, and his genius for organisation, which combined to render his public services so valuable, benefited by their exercise during the apparently fruitless and certainly irksome years of King's business life.

With the utmost affection and regard for his uncle, King never threw his heart into the business of the firm. As a small boy he had shown, in country walks with his father, all the interest of a child in birds and flowers with more than the usual power of remembering their names and peculiarities. But in the absence of parental stimulation and in spite of delicate health, this interest in natural objects, in place of being inhibited by the literary drill of a classical school, gradually developed into an overpowering taste for scientific study in general, and for zoological and botanical pursuits in particular. His innate ambition was to be a naturalist; his accidental attachment to a business failed to suppress his devotion to natural study. His spare time was given to field excursions; his enthusiasm gained him the "freedom" of the arcana of local nurserymen. His pursuits led King to introduce into the business premises specimens in which he was interested. These his uncle contemptuously termed "scroggs"; King's addiction to their study caused the worthy man genuine grief and much indignation. After his eighteenth year King's general health greatly improved, and it was clear that the situation could not persist. King continued in business until he reached his majority, but immediately thereafter he announced to his guardian his decision to devote what remained of his patrimony to acquiring a medical education, then the only avenue to a scientific career. His uncle, so far from expressing surprise, gave his approval to this decision, and thereafter did all in his power to further his nephew's designs. The relationship between the two men was perhaps more sympathetic after King entered the University of Aberdeen in 1861 than it had been before.

King's gift of lucid expression and his aptitude for business may, like his instinct for literature and art, have been paternal inheritances. The origin of his faculty for observation seems more obscure. It may have been derived from the maternal side, for among his undergraduate contemporaries was a relative,* in whom, as a boy, the love of natural history was also strongly developed, though in this instance the capacity for close and accurate observation was ultimately applied in a somewhat different field.

How distinguished as a student King proved, may be gathered from the fact that his contemporaries, after his first medical session, changed the old agnomen "Tertius" to "Optimus." The improvement in health which had begun in his eighteenth year continued; he had still to exercise unusual care, but the close of his university curriculum found him in more robust health than at its commencement. His capacity attracted the attention of all his teachers; those who exercised most influence upon him were the Professors of Botany (Dickie), Materia Medica (Harvey), and Anatomy (Struthers). King attended Dickie's class in 1861, and in 1862, when Dickie was incapacitated by illness, King was assistant to the deputy-professor, Dr. Dickson.† In 1863, when Dickie resumed work, and again in 1864, King continued to be assistant in the botanical department, and the attachment of the two men only ended with the death of the gentle and distinguished Dickie in 1882. The question as to the career King should adopt already exercised his chief and himself. King's predilection was towards cryptogamic botany; this was no doubt encouraged by so able an algologist as Dickie, who applied for counsel to Sir W. J. Hooker. Hooker's suggestion was that King should follow the example of his son, Dr. (now Sir J. D.) Hooker, and join the Naval Medical Service. But, while studying Materia Medica, Royle's 'Manual' led King to that author's other works; these, with Thomson's 'Narrative' and Hooker's 'Journal,' induced a desire to serve in India. There seemed little hope of this, recruitment of medical men for India having been suspended since 1860. But in April, 1865, the Indian Medical

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* Dr. James Rodger, whose father was an able mathematician and a successful man of business, and whose mother was a sister of King's mother, was one of King's class-fellows as a medical student. He graduated in 1865 along with King, and, like King, with highest academical honours. An accurate anatomist and a sound pathologist, Rodger was Senior Demonstrator of Anatomy under Prof. (afterwards Sir John) Struthers at Aberdeen from 1866 till 1871, and was Pathologist to the Royal Infirmary there from 1869 till 1886, when he was appointed a physician to the institution, and continued a member of its staff until his death in 1900. But the evidence is not conclusive; we must assume that King's father was at least capable of appreciating his child's interest in natural history, and we know that Rodger's father was an intimate friend of Prof. Dickie, F.R.S., enjoyed a considerable reputation as a local botanist, and took a keen interest in scientific matters generally.

† Alexander Dickson, afterwards Professor of Botany at Trinity College, Dublin (1866), Glasgow (1868), and Edinburgh (1879).

Service was reopened and a son of Prof. Harvey, already in a sister service,* threw up his commission and entered the Indian one. King decided to follow young Harvey's example; after attaining his M.B. in 1865, he joined the Indian Medical Service on October 2 of that year, and having spent the winter at Netley, left for India in the following March. Before embarking King paid a visit to Kew, and on the voyage was able to render the earliest of his many services to that establishment and to India. Dr. Hooker entrusted to his care the first medicinal Ipecacuanha plant sent to India; this King delivered in safety at the Royal Botanic Garden, Calcutta, when he reached that port on April 11, 1866.

On landing, King was attached to the General Hospital, but on May 9 he was transferred to the Medical College Hospital, Calcutta, and was appointed house surgeon there on May 18. This post the principal advised him to resign on August 20; in the performance of his duty he had contracted fever, followed by an attack of pneumonia, which endangered his life and threatened to light up the phthisical tendency of his boyhood. Already his work had commended him to his seniors, who used their influence to procure his transfer to the drier and healthier climate of Upper India. He was posted to military duty on August 29, and reached Agra on September 4, when he was attached to the 41st Bengal Infantry. On December 13, 1866, he again fell ill, and on returning to duty was given medical charge, on January 9, 1867, of the civil station of Muttra; but a month later his administrative chief, again with the object of promoting his recovery, procured his transfer to the still drier climate of Central India, where, on February 17, he took over medical charge of the 1st Central India Horse at Goona. This post he held till December 4, when he was transferred to the Political Department in Rajputana, taking up his duties at Deoli, in Marwar, on December 24, 1867. During the following year he served there and at Mount Abu, and afterwards at Jodhpur, but early in September he was selected to officiate as Superintendent of the Botanic Garden at Saharanpur, a post which he filled from December 9, 1868, till November 22, 1869. As his temporary appointment at Saharanpur drew to an end, King found himself at the parting of the ways. His energy and ability impressed all those with whom he came in contact, and some glimpse of his organising faculty had been given in connection with famine work in Rajputana. The medical authorities were anxious to secure his services as a Deputy Sanitary Commissioner, and orders to take up civil medical duty on his relief at Saharanpur were issued. But the chief political officer in Rajputana had been equally impressed, and, having learned that the Forest Department was in need of competent officers, strongly advised the Conservator in the North-west Provinces to ask for King's services. King was accordingly invited in September, 1869, to accept an Assistant Conservatorship. The forest appointment offered greater scope for the utilisation of his botanical knowledge, and was accepted by King, who was thereupon placed in charge of the Dehra Dún forests, taking up his new duties on December 3, 1869.

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* Dr. R. Harvey, C.B., D.S.O., subsequently Director-General, Indian Medical Service, in which capacity he died at Simla, Panjab, December 1, 1901.

At Agra, Muttra, and Saharanpur King laid the foundation of that knowledge of the plants of the Upper Gangetic Plain, shown in a contribution by him to the 'N.W. Provinces Gazetteer.' At Goona the flora of Central India did not suffice to occupy all his leisure; the balance he devoted to an ornithological survey of the district and to the preparation of a series of skeletons for the anatomical museum of his university. This work he continued in Rajputana, where he also made large botanical collections, investigated the plants used as food in times of scarcity, and studied the vegetation from what is now termed the œcological standpoint. At Saharanpur he found time, amid his administrative duties, for morphological and economic botanical studies.

The forest work at Dehra Dún gave King freer scope for the display of his powers. Zeal, energy, and candour were in him combined with a charm of manner which attached to him those with whom he had dealings. He could direct without damping the enthusiasm of those who served under him, while commanding the confidence of those under whom he served. Honest difference of opinion he seemed to find attractive; his anger never was provoked save by attempts at intrigue or subterfuge. These qualities were sorely needed in the Assistant Conservatorship with which he was entrusted. The situation he had to face is disclosed in a 'Memorandum on the Dehra Dhoon Government Forests,' presented to Government in April, 1871. To help him in his task he had been invested with the powers of a subordinate magistrate of the first class within forest limits on March 18, 1870; in performing it he virtually saved these forests from extinction. The nature of his achievement during the fifteen preceding months may be gathered from a judgment delivered on May 21, 1871, of which the following passage forms part:—"I do not think it would be right of me to close this case without putting on record some expression of opinion regarding the zeal displayed by Dr. King, and the debt which the Forest Department owes him. For years a most complete system of bribery and corruption had been going on in the Forest office; Government property to the value of thousands of rupees had been yearly stolen with the connivance of the Forest officials. Dr. King had hardly been in office a month when he saw how affairs stood, and before the end of six months he had obtained convictions against the principal offenders. The amount of labour Dr. King had to go through to obtain this satisfactory result is beyond belief. Day and night he never rested; through the most impenetrable jungles in the most unhealthy season he forced his way. No man, woman, or child who could throw any light on the subject was left unseen and unquestioned; once he got a clue he never let it drop; account-books and papers sent into the heart of foreign territory to be beyond grasp he ferreted out and laid before the Court. The result is that he has thrown light on all the most secret transactions. The character of all the officials in the Forest Department has been clearly portrayed; the various ways Government had hitherto been defrauded and robbed have been found out and exposed; if Dr. King's successors do not take advantage of this it is their fault. The benefit which should accrue to the Forest Department by Dr. King's labour, if followed up in a proper spirit and with ordinary energy, is incalculable."

Little more than a year after joining the department King was appointed, on January 28, 1871, to officiate as Additional Deputy Conservator in charge of the Kumaon Forest Division, and on March 2 he was recommended for permanent promotion to this higher grade. While acting as Deputy Conservator King was ordered to prepare a "Report on Forest Conservancy, etc., for Raneekhet," one of the N.W. Himalayan hill-stations. This report he submitted in June, 1871, and its nature may be gathered from the 'Gazette' of India for September 9, which officially states: "That the Governor-General in Council has read Dr. King's very interesting report with great satisfaction, and cordially endorses the praise bestowed upon it by His Honour the Lieutenant-Governor. The recommendations contained in it are excellent, and His Excellency in Council trusts they will be borne in mind and carefully carried out." King further made a careful study of tea-pruning under the conditions that prevail in the Kangra Valley, and although it had reference primarily to the N.W. Himalaya, a successful tea-garden manager in N.E. India once remarked of King's paper that "before reading it he had pruned with his hands, after its perusal he could prune with his head." During his forest service King formed extensive botanical collections, and was acquiring the knowledge displayed afterwards in his 'Gazetteer' "List of the Plants of Garhwál, Jaunsár Báwar, and Dehra Dún."

The recommendation that King should be made a permanent Deputy Conservator was accepted by Government. Owing, however, to there being no vacancy in the N.W. Provinces, this promotion, it was decided, must involve his transfer to Burma. But before this arrangement could be carried out King's forest service came to an end. Dr. Thomas Anderson, Superintendent of the Royal Botanic Garden, Calcutta, and of Cinchona Cultivation in Bengal, fell a victim, in 1869, to the energy and zeal with which he had for years been labouring to establish plantations of cinchona in Sikkim. He had to be invalided to Europe, where he died in October, 1870. On March 10, 1871, the Secretary of State for India selected King as successor to Anderson (India Office despatch, March 23; Government of India Order, May 22). On being relieved of his forest duties King left for Calcutta and entered on his new charge, which included the Professorship of Botany in the Medical College of Bengal, and where the tasks before him were heavy ones, on July 10, 1871.

Two cyclones of extreme severity which swept over Calcutta in 1864 and in 1867 had ruined every park and garden in the neighbourhood. The Botanic Gardens, formerly famed for possessing one of the finest collections of trees in the East, had been reduced to a comparatively naked plain, thoroughly exposed to sun and wind, and therefore favourable to the growth of rank grasses which smothered the young trees and shrubs planted to replace the uprooted veterans. Roads and paths were insufficient in number and unsatisfactory in condition and alignment. The site of the garden, being part of the rice-swamp which forms the Gangetic delta, was far from suitable to the successful cultivation of many desirable indigenous and exotic species. The residences of the garden employés, native and European alike, were inadequate and insanitary. The accommodation for the herbarium and library was cramped and inconvenient; the herbarium collection, though extensive, was very unequal. The labours of Roxburgh, Buchanan, and Wallich from 1793 to 1828 had brought together the richest botanical collection hitherto made in Asia. But in 1828 this collection was taken to Europe, and Wallich, then on leave in England, dispersed it on behalf of the East India Company with a generosity so lavish that nothing was left for the institution at whose cost and on whose behalf it had been formed. Something was done to repair this injury by Wallich himself on his return (1832—46), by Falconer (1846—54), by Thomson (1854—59), and by King's predecessor Anderson (1859—69), while the generous aid of Kew had provided Calcutta with a substantial share of the contents of the East India House cellars. But great leeway had still to be made up in order to render the Calcutta Herbarium commensurate with the needs of so important a botanical centre.

The problems connected with cinchona were of equal importance and of even greater difficulty. The Sikkim plantations, begun by Anderson ten years before, and pushed on with a zeal which cost that indefatigable officer his life, were an established fact when King assumed control. The policy of Government had been to act, as in the case of tea, only as a pioneer. So soon as it could be shown that private growers were in a position to undertake the enterprise, Government was prepared to dispose of these experimental plantations and retire from the field. In the case of cinchona this policy could not be carried out. The experience of these ten years had proved that in spite of every encouragement and assistance, the cultivation of cinchona in Northern India is, owing to natural causes, unprofitable to private enterprise. Government itself had therefore to attempt the economic separation, from the bark produced on its own plantations, of the alkaloids this bark contained, and to utilise these alkaloids in combating the ravages of malaria. Attempts in this direction had been made before King assumed charge; these attempts had not been attended with success. But there was another equally important problem to be dealt with. The bark of those cinchonas that had so far been most successfully grown in Sikkim and that were therefore chiefly represented in the Government plantations, is bark that, while rich in total alkaloids, is relatively poor in quinine, the most important of these alkaloids. It was therefore King's ambition to replace the kinds then most largely cultivated by others whose bark is rich in quinine, and eventually to separate this quinine in such a manner as to obviate financial loss to Government. With characteristic energy King attacked these problems: as regards the first, urging the employment of a competent quinologist; as regards the second, himself attempting its solution on the spot. His zeal almost involved him in the fate that had befallen Anderson. Exposure and fatigue during his work on the plantation induced severe illness, which became aggravated while on an official visit to the Madras plantations in July, 1872. He developed symptoms of phthisis, and from July 16 was placed on the sick list at Coonoor. A month later his condition was so serious that he was invalided to Europe, and his friends hardly ventured to anticipate his return. After a year, spent mostly on the Riviera, his health, however, became so improved that he was able to resume his duties on November 5, 1873.

In 1874, King obtained the approval of Government for the improvements required in the Botanic Gardens as a scientific centre and a place of public resort. His designs involved prolonged work and considerable outlay. The funds required could only be gradually allotted; that they were granted at all gives ample proof of the enlightened liberality of the Bengal Government and the confidence which King's administrative gifts inspired. During the next nine years the Gardens were practically reconstructed. By excavating a series of lakes and ponds, so designed as to produce a variety of pleasing effects, sufficient soil was obtained to raise the level of the whole of the grounds. These sheets of water were connected by underground pipes, and the whole system was so arranged as to be kept at a uniform level by pumping water from the contiguous river. Many footpaths and carriage roads were laid out so that visitors can drive to any part of the garden. Elegant conservatories and a noble palm-house were built. New potting sheds, tool stores, and propagating pits were supplied and good dwelling-houses were provided for the members of the garden establishment both native and European. A handsome fireproof herbarium, on the lines of that at Kew, was erected to accommodate the rapidly-growing collection of dried plants and the valuable library. Minor improvements were added in subsequent years, but by 1883 the heaviest of King's garden work was over.

While effecting these improvements, King steadily added desirable species, indigenous and exotic, to the collections of living plants. Whenever this was compatible with the health of the plants and the production of pleasing effects King arranged his species with regard to their affinities, so that one part of the great garden can boast its fine palmetum, pandanetum, bambusetum; elsewhere other natural groups are similarly treated. But King had all the horror of the true lover of plants for a pedantic arrangement of species in rectilinear blocks according to what are conventionally termed natural families and regardless of the conditions suited to particular species. The first duty of a gardener he held to be the proper culture of his plants; the needs of the species grown, not the pragmatical requirements of the methodist, were his chief consideration. Here and there he aggregated with the happiest results groups of species from some particular geographical area, thus reproducing plant associations which, though unmeaning except from the artistic standpoint to the average European, are readily appreciated by intelligent native visitors. His operations were controlled with a singular prescience for ultimate effects and his years of unremitting toil were amply rewarded. The whole place bears the impress of King's influence and care, and the charm and beauty of its lakes and groves, its avenues and lawns for which the Royal Garden at Calcutta is now so justly famed, serve as an adequate memorial to his energy, patience and skill as a landscape gardener.

King's work in the Botanic Garden by no means exhausts his achievements in this direction. Shortly after his return to India in 1873 the amenities of Calcutta were enhanced by the addition of a zoological garden. King was appointed an original member of its Committee of Management, the Lieutenant-Governor in person serving as President. The site selected was occupied by a collection of miserable native huts; in a few years, under King's skilful guidance, it became one of the most attractive public resorts in India. Very soon afterwards the site of a summer residence for the Lieutenant-Governor was acquired at Darjeeling. The demesne in which the mansion stands was laid out under King's eye and the consummate art with which he employed the constituents of a natural forest and blended the effects produced within the area treated with those to be obtained from adjacent hill slopes and distant views has rendered these grounds at once the ideal of what such a place should be and the despair of those who would repeat the results. Again, in 1879, when, with the help of private munificence, Government was able to provide at Darjeeling a temperate annexe to the Botanic Garden at Calcutta, King was given administrative charge of this new garden. By a combination of the methods employed for the "Shrubbery" grounds at Darjeeling with those applied to the Zoological Garden at Calcutta, King in a few years created another place of public resort at once beautiful and instructive.

When King resumed charge of the Cinchona Department in 1873, he found that a quinologist had been appointed; he was, therefore, for the moment relieved of his anxiety with regard to factory operations. The officer appointed, Mr. C. H. Wood, devised a satisfactory method of extracting the mixed alkaloids from cinchona bark, and to King fell the delicate duty of creating a market for the resulting product, known as Cinchona Febrifuge. He brought to this task all the qualifications of an expert business man, surmounting every difficulty, and firmly establishing the distribution of the article on commercial, as opposed to eleemosynary, lines. But on October 13, 1877, Wood was transferred to Calcutta and King was placed in administrative charge of the factory, and in August, 1879, Wood, for domestic reasons, resigned Government service. When Wood retired he had not yet devised an economic method of separating quinine; he had, however, left his process for extracting febrifuge in excellent working order. In Mr. J. A. Gammie, the resident manager of the plantation, King had an able and resourceful lieutenant, who had worked in conjunction with Wood and was thoroughly competent to conduct Wood's febrifuge process.

Difficulties due to natural causes at first impeded King's substitution of the cultivation of yellow, or quinine-yielding, cinchonas for that of red cinchonas, in which the proportion of quinine is low. But for the skilful co-operation of Gammie these difficulties must have proved insurmountable, though, great as they were, they proved trifling as compared with other difficulties which only the confidence that he inspired in his immediate superiors enabled King to defeat. From the same source came difficulties connected with manufacture. When Wood resigned the quinologist's post in 1879, King had been deputed to Java to study the working of the Dutch cinchona department in that island. When he returned to India, on December 5, 1879, King found himself appointed to act as his own quinologist. The situation, for one whose passion was for thorough work and yet who was not himself an expert chemist, was full of difficulty; but the situation had to be faced, and he faced it with courage. Wood, after his return to England, took the keenest interest in the work, striving in his own laboratory to master some economical mode of obtaining quinine, while Gammie made trial of his suggestions on a commercial scale in the factory in Sikkim. Eventually King himself conduced to the ultimate success. He spent the summer of 1884 on furlough in Europe. Botanical studies on which he was engaged necessitated a visit to the Dutch herbaria. While in Holland he acquired some valuable information as to a process for separating quinine, which he at once made known to Wood, who was thereby led to devise a process more hopeful than any previous one. Gammie was able to visit England on furlough in 1885; he studied the details of the new process in Wood's laboratory, and on his return to India found that it was practicable on a commercial scale. The separation of pure quinine on the spot without involving financial loss to Government was at last possible, and by the end of 1887 a factory had been established and the manufacture of quinine on commercial lines was in full operation. In reporting this event, King was content to recount "the generous way in which Mr. Wood, without any pecuniary reward, initiated and invented it [the process] in his private laboratory, while Mr. Gammie perfected it in the Government factory. Without Mr. Wood the process would not have been invented, while without Mr. Gammie it would not have been successfully applied to manufacture."

The achievement was, after all, only a step towards the realisation of the original design of Government to supply the people of India, on a self-supporting basis, with quinine at a nominal cost. The attempt to supply the article on an eleemosynary basis had, indeed, already been made, police outposts being utilised as the distributing agency. But this humane effort was promptly defeated by small capitalists, who bought up the whole supplies as soon as these reached the various outposts, in order to resell the drug at a handsome profit and yet at rates which undersold the regular vendor. A firm of European merchants, inspired partly by genuine philanthropy, partly by a legitimate desire to extend their business, had also essayed the task, but had been compelled to abandon it, owing to the impossibility of organising a reliable distributing agency. Clearly, therefore, no special agency could be economically created: some already existing one must be utilised. Clearly, too, the drug must be sold at a rate which, while securing Government against loss, should at the same time eliminate temptation on the part of outsiders to exploit the humanity of the authorities. King saw at a glance how the desired result might be attained, but the confidence he inspired in Government was not in itself sufficient to ensure the success of his design, which demanded not only the consent but the enthusiastic support of other heads of departments. In gaining this support the charm of his personality was as effective as it had been in securing the co-operation of his colleagues. The scheme involved the sale at every post-office of Government quinine, made up in doses of five grains each. Each dose was to be enclosed in a neat sealed packet. Each packet was to be sold for one farthing, and, together with brief instructions in the various vernaculars, was to bear the Royal Arms as a guarantee of genuineness. To encourage postal officials to push sales, a small commission was to be allowed, and facilities for replenishing stocks were to be offered. To eliminate the risk of adulteration and pilfering, the making-up of the packets was to be entrusted to the Prisons Department, who would receive the quinine in bulk direct from the Cinchona stores. In perfecting the scheme, King worked in co-operation with the Financial Secretary to Government, Mr. (now Sir Herbert) Risley. But the Postmaster-General for Bengal, Mr. Kisch, devised the procedure regulating the actual sales; the ingenuity of the Superintendent of the Alipur Jail, Mr. Larrymore, hit upon a method of cheaply, rapidly, and accurately dividing the quinine into doses, and the expert advice of the Government Printer, Mr. Lewis, guided the details connected with the preparation of the envelopes. The success of the scheme depended on the precision with which each department did its share of the work, and on the accuracy of the calculations with regard to the cost of each operation. These calculations had of necessity to be so close as to leave no margin; an error at any point might easily have involved financial loss. The scheme, fully matured, was put into operation in 1893, and worked from the outset without a hitch. To the officers of these co-operating departments King attributed the success, after thirty years of effort, of the design enunciated by Government when it introduced cinchona to India:—"To put the only medicine that is of any use in the cure of the commonest and most fatal of Indian diseases within the reach of the poorest."

In 1874 King also commenced on a definite plan, the details of which he wisely subordinated to current exigencies, a survey of the vegetation of the countries within the sphere of influence of the Calcutta garden. These include the Eastern Himalaya, Bengal, Assam, Burma, the Andaman and Nicobar Islands, and the Malayan Peninsula. His first object was to fill up gaps in the Calcutta Collection rather than to investigate afresh areas already examined; to this end he sent independent collectors to unvisited districts or attached them to military expeditions or survey parties. But his efforts were largely aided by personal friends, and in no branch of his work was his magnetic influence more potent than in this. He imparted to officers of Government, both civil and military, to missionaries, planters and travellers some share of his own enthusiasm, and many of the most valuable additions to the Calcutta herbarium were the result of his endeavours in this direction. His multifarious duties left him few opportunities for personal travel, but he never allowed them to impede his constant supervision of the work of his botanical artists. He was thus enabled to bring together a collection of specimens and drawings far surpassing in extent and value that dispersed in 1828, and to take a considerable share in the task of supplying material for the use of Sir Joseph Hooker, while that botanist was engaged from 1872 to 1897 in preparing the 'Flora of British India.' It was therefore fitting that when, in 1891, the botanical officers serving in the different Presidencies were linked together in one department, King was appointed the first Director of the Botanical Survey of India. In this capacity he urged the necessity for the preparation of a series of local or regional floras to supplement Hooker's great work. His proposals, after being approved alike by the local governments concerned and by the Supreme Government, encountered difficulties akin to those he had experienced in connection with cinchona, so that nothing beyond what he himself could accomplish had been done in this direction when he left India in 1898. In the end these difficulties were overcome, and the work he had shown to be necessary has already been partly accomplished.

As Professor of Botany at the Medical College of Bengal, King was a lucid and effective teacher, and in the course of study to which he subjected his pupils he, with the approval of Government, effected at the outset alterations which to his practical mind seemed improvements. The course, as he found it, was modelled on those adopted in medical schools in Britain, where the teacher was either content to coach his students to the point required to enable them to pass an examination on some prescribed standard, or was prone, if enthusiastic, to endeavour to bring his pupils to some approximation to his own standard of botanical knowledge. The first method King held to be a waste of the time both of teacher and taught; the second, even if the laws of supply and demand had rendered it desirable, he found to be impossible. His students, with hardly an exception, were young men who had suffered from what he held to be the injurious incubus, a western literary education; with minds often originally bright, their natural powers of observation had been inhibited and sometimes atrophied by close attention to the written word. In consideration of the fact that the real purpose of their presence in college was to acquire a practical knowledge of surgery and medicine, he deemed it his duty to treat the subject he taught as purely ancillary to this laudable end. His teaching therefore resolved itself, not into a course of Botany in the ordinary acceptation of the term, but into a steady application of botanical facts and truths to the training of the various senses of his students. If in the end they did come to know a good deal about the subject, this King considered a purely incidental result. The object he strove, and strove successfully, to attain was to habituate his pupils to the art of observing natural facts, and to accustom them to the ordeal of giving reasons for the faith that was in them when confronted with objects that, though similar in externals, were essentially different.

The exercise of King's business capacity was not limited to the departments which he administered. His local Government appointed him a member of the visiting Board of the Bengal Engineering College, an institution with whose objects as a technical school he was in entire sympathy, and in whose progress he took a warm and effective interest. He was appointed by its Chancellor a Fellow of the University of Calcutta, and was long a trusted member of the Senate, for a time also representing the medical faculty on the Syndicate. He represented the Supreme Government as a Trustee of the Indian Museum, an institution for which he did much important work, especially during a number of years when he was Chairman of the Trustees; from his resignation of that office till his retirement from Indian service he was Vice-chairman. When in 1894 the Government of India organised an enquiry into the indigenous drugs of the country, King was appointed Chairman of the Central Committee, and served in this capacity till he left for Europe. He took a deep interest in the welfare of the Asiatic Society of Bengal, and although he did not often accept a seat on its Council, he was always the trusted adviser of the Society's officers in matters relating to its natural history side. He was an active member of Council, and at one time President of the Agricultural and Horticultural Society of India.

It is somewhat interesting to observe that, in spite of his early distaste for business, King's public services should have derived their chief value from his remarkable business aptitude, and that although the extent and gravity of his official duties did not prevent the simultaneous prosecution of purely scientific studies, the possession of this business aptitude deprived him for many years of any opportunity of presenting ordered statements of his results. It is equally interesting to find that, as regards his scientific work, the line which he took was not that towards which his tastes naturally led. When King reached India in 1866 his botanical interests were centred on physiological and morphological problems, and especially on those connected with cryptogams. Here, again, circumstance proved stronger than predilection. The comparative poverty of the floras of Central India and Rajputanà led him to expend his surplus energy in important zoological studies; during the rest of his career these were given to systematic work connected with flowering plants. His practical mind realised, from the time he took charge of the Saharanpur garden, that however enticing his favourite studies might be, the path of duty for him led elsewhere; that the immediate needs of people and Government alike demanded that every official Indian botanist should devote himself to aiding Hooker in the prosecution of his fundamental undertaking of providing recognisable descriptions of Indian phanerogams; and that until this floristic study was completed, the time for indulging in the work he personally preferred had not yet come. His duties as a forest officer taught him how difficult and yet how essential the recognition of species that are of economic importance may be ; his experience then and afterwards, when engaged in the formation of a first-rate herbarium collection, led him to realise how frequently competent field workers, whose results in obtaining material for the study of herbaceous plants or shrubs may leave nothing to be desired, are deterred by what are doubtless serious physical difficulties from supplying specimens that adequately illustrate arboreous types. His sense of the extreme importance, from the industrial standpoint, of full accounts of the constituents of the Indian forests, led him by precept and example constantly to strive to remedy this well-known defect. With all this he fully realised the desirability of advancing our knowledge of Indian cryptogams, more especially in regard to their connection with pathological problems, but he failed, for once, to convince Government how desirable it was to add a competent cryptogamist to the garden staff. He did what he could to remedy the defect by referring material to experts in Europe, and here again his personal influence was of incalculable benefit to India. A friend, Dr. Cunningham,* devoted much of his scanty leisure to questions connected with vegetable pathology ; another friend, Dr. Barclay,† was an ardent student of the life-histories of the Uredineæ. For many years these two workers dealt on King's behalf with critical references relating to the field of study which King was precluded from entering and their generous co-operation with him in the public interest only ended with the departure from India of the one and the untimely death of the other. Throughout his active career King kept himself abreast of what was done in most branches of botanical activity, but intimate friends alone were aware of the pain it gave him to observe the gradual drifting apart of workers in different lines of research. What grieved him most was the hostility, especially when this was veiled, sometimes displayed by men whose work connected with what they termed "scientific" botany he held in high regard, towards "systematic" botany. This attitude on the part of students of problems which naturally attracted himself, towards conscientious workers in the field to which circumstance and a sense of duty confined him, caused King deep distress.

The fact that King's scientific attainments were on a level with his administrative gifts, though unknown to the world at large, could not be concealed from those with whom he corresponded on botanical subjects, and in 1884 his university conferred on him the degree of LL.D., while in 1887 he was elected into the Royal Society. He had since 1874 in reality done much critical work, but it was not until 1887, when the progress made with his garden improvements and especially in connection with the manufacture of quinine seemed to justify the step, that King commenced the publication of important contributions to botanical literature. In that year the enlightened liberality of the Government of Bengal enabled King to found the 'Annals of the Royal Botanic Garden, Calcutta,' a series of sumptuous volumes in which he proceeded to publish amply illustrated monographs of difficult and unwieldy genera and families. The first of these deals with "the species of Ficus of the Indo-Malayan and Chinese countries." On this work he had bestowed the labour of much of his scanty leisure for eleven years, during which time he had examined the material preserved in every important European collection. The objects he had in view were altogether practical ones; the genus was selected because of its being largely composed of trees, many of them being of economic importance, and the monograph was primarily intended to break ground for Sir Joseph Hooker and to assist that author in subsequently dealing with its species. The work, however, is marked by such accuracy, lucidity, and completeness that it at once placed King among the foremost systematic botanists of his time, and its appearance was rapidly followed by that of equally finished works on Quercus, Castanopsis, Artocarpus, and Myristica, all prepared with the same object and selected for the same reasons. When King visited Java in 1879 he had an opportunity of seeing something of the rich vegetation of Malaya, which made on his mind an ineffaceable impression. From Singapore he paid a botanical visit to Johor, in company with his friend Archdeacon (now Bishop) Hose. He collected personally in Penang and Province Wellesley, and was subsequently able to arrange for the systematic botanical exploration of Perak. In 1886 facilities were afforded, at the request of King's friend, Sir Hugh Low, to Father Scortechini, who had also made extensive collections in Perak, to commence the preparation in the Calcutta herbarium of a flora of that State. Scortechini, unfortunately, soon afterwards died, bequeathing to the Calcutta Garden all his specimens, drawings, and notes. Sir Joseph Hooker and Sir Hugh Low now begged King himself to undertake this very urgent task, and in 1889 he commenced single-handed a floristic study of the whole Malayan Peninsula. As preliminary to the preparation of a local flora of the region—the first of the series of such floras, whose publication for the various provinces of India he was two years later officially entitled to urge—King began to issue, in the 'Journal of the Asiatic Society of Bengal,' a series of contributions intended to serve as "Materials for a Flora of the Malayan Peninsula," but prepared with such care that they form a satisfactory substitute for a final work.

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* D. D. Cunningham, C.I.E., F.R.S., Professor of Physiology, Calcutta, and Secretary to the Sanitary Commissioner with the Government of India.

† Arthur Barclay (1852—1891), Secretary to the Director-General, Indian Medical Service.

Five instalments of this great undertaking, completing the Thalamifloræ, were issued up to 1893, but in the case of two important families, Magnoliaceæ and Anonaceæ, the study of the Malayan forms involved a careful examination of extra-Malayan material, the results of which were embodied in two great monographs simultaneously published in the garden 'Annals.' In 1895, King, having attained the age of fifty-five, was, under Indian rules, due to retire on April 11 without being able to qualify for the pension payable after thirty years' service. In consideration of the importance of the work he had in hand, his service was extended for two years, and on July 1, 1895, he was further permitted to resign his chair at the Medical College so as to leave more time for the floristic work on which he was engaged. In 1896 with the eighth part of the 'Materials,' King completed the Discifloræ, and in 1897 he was granted a further extension to permit him to carry still further his Malayan work and to complete a sumptuous monograph of the 'Orchids of the Sikkim Himalaya,' of great importance to horticulture, for which he provided the text, while one of his Cinchona officers, Mr. R. Pantling, prepared the illustrations. Towards the end of 1897 his health, which since 1873 had been uniformly good, was completely undermined by a severe attack of fever, and his medical advisers peremptorily ordered the termination of his service. But before he left India on February 28, 1898, after more than thirty-two years of devoted service to the people and the Government, he had the satisfaction of seeing the issue of the orchid monograph, and had carried his Malayan work to the middle of the Calycifloræ, at the end of the tenth fasciculus. On reaching England, King, resumed at Kew, his work on the Malayan flora. The state of his health, however, prevented his making great progress during 1898, and in 1899, owing to his consenting to serve as President of the botanical section of the British Association at its meeting at Dover, he was able to accomplish less than he had hoped. He had, moreover, under medical advice, to spend each winter and spring on the Riviera, and soon realised that he might never finish the task he had allotted himself. He faced the contingency with characteristic practicality. By arrangement with his friend, Mr. H. N. Ridley, Director of the Singapore Botanic Garden, that botanist undertook the elaboration of the Monocotyledonous families, while King worked out the remaining Dicotyledons, and when, in 1902, with the issue of the thirteenth part, King had finished the Calycifloræ, he was joined by his friend, Mr. J. S. Gamble, in the elaboration of the Corollifloræ. For three more years King took his full share in the joint work, which now made rapid progress; after 1905 partial loss of sight and progressive infirmity led to his enforced abandonment of active participation in the task, and the only share he could take in the preparation of the twenty-first part, whose issue coincided almost to a day with his death and completed the Corollifloræ, was the examination of the sheets as they passed through the Press. He had, however, the satisfaction of seeing the issue in 1907 of the first portion, and the completion in 1908 of what remained of Mr. Ridley's contribution to the great undertaking begun in 1889.

King's reputation as a landscape gardener was well known; it brought him honorary association with various horticultural societies and was recognised by the award of the Royal Horticultural Society's Victoria Medal in 1901. The value of his services to humanity in connection with the separation and especially the distribution of quinine brought him honorary membership of the Pharmaceutical Society, the grade of Officier d'Instruction publique, the gift of a ring of honour by the Czar Alexander III, and the Companionship, in 1890, of the Order of the Indian Empire. His work as a systematic botanist was also widely appreciated; he was a corresponding member of the Bavarian Academy, an honorary member of the Royal Botanic Society of Belgium and of the Deutsch Botanische Gesellschaft, one of the six honorary British Fellows of the Botanic Society of Edinburgh, and, a distinction that gratified him more than any other, an honorary member, after he left India, of the Asiatic Society of Bengal, with which he had been connected since 1867. The University of Upsala presented him with a medal in recognition of his botanical studies, and the Linnean Society, to which he had been elected in 1870, awarded him its Linnean medal in 1901. On January 1, 1898, he was, in recognition of his long and distinguished service, created a K.C.I.E., and immediately after his retirement a number of his personal friends united in obtaining a medallion portrait in bronze, which was placed, with a similar portrait of his friend, Dr. Cunningham, who for many years was Secretary to the Committee, in the Zoological Gardens which King had designed. A replica of King's portrait was placed in the Royal Botanic Garden whose beauty he had restored. At San Remo, where he wintered yearly from 1898 till his death, another memorial, connected with a public institution whose welfare he had much at heart, will bear lasting witness to his quiet but effective devotion to the cause of practical philanthropy.

King married, in 1868, Jane Anne, daughter of Dr. G. J. Nicol; during his illness in 1897 she was with him in India. As he was slowly recovering, Lady King's health gave way. On the homeward voyage she gradually sank; she died in London the day following their arrival in England. From this blow King never fully recovered; its effect became more and more apparent as the solace of strenuous work was denied him. The hæmorrhagic tendency of early life reasserted itself, and led to the rupture of a retinal vessel which deprived him of the use of an eye. The tendency steadily increased, and the melancholy induced by the feeling that his days of usefulness had ended was mercifully relieved by an apoplectic seizure to which King succumbed at San Remo on February 12, 1909. His remains were interred, as he had desired, where he died.

King's wide knowledge, which extended to most branches of science and embraced many aspects of art and literature, was accompanied by a natural modesty and a personal charm that rendered intercourse with him extremely pleasing, though literary or artistic friends rarely came to know of his scientific tastes, and scientific acquaintances had still fewer opportunities of appreciating his critical acumen. But these, and other friends outside either category, fully understood his innate goodness and courtesy, his transparent candour, his shrewd sense, and his keen but kindly wit. A wise counsellor and an unfailing friend, he was loved by all who were privileged to know him. King's life was spent in doing with his might what his hand found to do, and if others have made more striking contributions to natural knowledge, none have rendered more self-sacrificing and devoted service to the nations of India and to the science of Botany.

D. P.

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